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CONCERNING FLUCTUATIONS IN POPULATIONS OF THE PROLIFIC AND WIDELY DISTRIBUTED MUSKRAT[1]

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Even among rodents, the muskrat, *Ondatra zibethicus* in its 16 described subspecies, is notable for its fecundity; and its importance in the fur trade has focussed a great deal of public attention on its numerical status. Although its annual fluctuations may often show neither the extremes nor the comparative rhythmicity of the fluctuations of some of the allied voles (*Microtus spp.*), the muskrat is a mammal that *can* vary decidedly in abundance from one year to another.

Since Johnson's (1925) work in New York, the muskrat has been investigated under free-living conditions perhaps as much as any subhuman mammal on earth—mostly as a fur-bearer in its native North America and its new range in northern Eurasia (Artimo, 1949), but also as an introduced pest in central and western Europe (Ulbrich, 1930; Storer, 1937; Warwick, 1940). The muskrat's psychological traits have not encouraged extensive laboratory experimentation, but the species living "naturally" has proved to be a suitable vehicle for the exploration of rather broad population phenomena.

Most of my own participation in these investigations may be said to have begun in Iowa in 1934, after an earlier (1915–28) background of experience as a hunter and fur trapper in marsh and stream areas of South Dakota and northern Minnesota.

The principal objective of the Iowa program of muskrat studies always has been to learn more about the rules of order governing the distribution and maintenance of populations in different types of habitat. In connection with the program, I spent approximately 25,000 hours on field research on the muskrat and its associated biota, in addition to supporting work in laboratory and library and on correspondence and manuscripts. "Key"

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data were sought from definite observational areas, the same places (totalling about 30 square miles) being regularly kept under observation from year to year. Emergencies, epizootics, population adjustments, or other events affecting the local or regional status of the muskrats were followed as closely as time and techniques permitted. The intensive work on observational areas was supplemented by enough travel outside of Iowa to gain some familiarity with representative parts of about four-fifths of the muskrat's geographic range in the United States and Canada. This out-of-state travel was substantially aided by special grants from the Wildlife Management Institute and by the excellent cooperation of the U. S. Fish and Wildlife Service, the Hudson's Bay Company, and the Manitoba Government.

As yet, comparatively little has been published on our results, except for preliminary treatment of specific topics (see, for examples, Errington, 1941, 1943, 1946). However, the area data in my possession recently have been written up and, together with introductory material and a review of the biogeography of muskrat subspecies, comprise the first three parts of a four-part book manuscript. The fourth part of the book, which is the synthesis, remains to be prepared, but the trends revealed by the data look sufficiently clear to warrant summarizing, especially in so far as they may illustrate differences between the fundamental and the incidental in population dynamics.

PHYSICAL ENVIRONMENT AND MUSKRAT DENSITIES

Apart from a few special divergences in behavior, such as certain western subspecies not building the usual lodges in marshy habitats and the drowning of *O.z. rivalicius* under ice in Louisiana (O'Neil, 1949), muskrats may be expected to respond to opportunities and crises in much the same way over their occupied range in the northern hemisphere. From beaver pools in mountain streams to desert waterholes, glacial marshes to irrigation seepages, river bayous to brooks, ditches, and lakes, the three widely distributed subspecies that I have worked with in detail (*zibethicus, cinnamominus* and *osoyoosensis*) live like muskrats anywhere, whether confronted by subarctic or subtropical extremes.

Within the limits imposed by its adaptations for a semi-aquatic existence, this rodent, with its peculiar combination of frailty and ruggedness, often demonstrates a remarkable ability to stay alive, to live somehow, at least at times, up to the very edges of what may be considered habitable range. Under stimulus of emergencies or population pressures, the species may be forced to overflow the better habitats, even to the extent of making massive, lemming-like pioneering thrusts into totally uninhabitable range.

It should not be quite a truism to state that the number of muskrats to be found in an area *partly* depends upon essential water, food, and shelter, and the relation of these to each other. A muskrat must have more than a will to live to endure nine months of alpine or arctic cold under several feet of ice. The species is responsive to good sources of food—corn fields bordering streams or heavy stands of cattails, bulrushes, and duck potatoes in

marshes—as long as its minimal requirements for space and water are met. Water, itself, may mean the difference between muskrats living and muskrats dying, but, in temperate regions, depths of a foot or two that the animals can depend upon are preferable to great quantities that may through sudden fluctuations, wave action, drowning of vegetation, etc., bring about other complications. Shelter may be afforded either by lodges built in marshes or by burrows dug in banks, or, during emergencies, by land retreats. The usually inferior grades of muskrat environment include large rivers or open lakes without much food, or streams or shallow sloughs that go dry except in wet weather. Among the superior places are tile-fed drainage ditches running through cultivated lands and lush marshes having bottoms well covered by fresh or brackish water.

One of the most influential factors in the lives of muskrats now recognizable from the population case histories on record is that of *density*, or the numbers of the animals that are *already* present in a habitat as the annual breeding season gets under way or as population adjustments occur in late summer and fall. Pearl and Parker's (1922) early conclusions as to the importance of the density factor in regulating populations have been substantiated in many ways by the muskrat data.

The functioning of the density factor in muskrat populations may or may not be attended by visible food shortages or by increased fighting or disease losses or by migrations. It may be seen to be both interlinked with and separate from the physical environment. Greater restlessness and a compounding of troubles are typical manifestations to be noted as densities rise past the levels that are well tolerated or that can be well accommodated.

On the whole, the long-term records from observational areas in Iowa and elsewhere reveal strong tendencies for spring-to-fall increases of muskrat populations to conform to mathematical formulas that *differ with the area* but which for a *given area* may remain *apparently unchanged* for years at a stretch.

At one extreme, very low densities may be expected to show low rates of increase between spring and fall. Survival rates of the young born of low populations living in good environment may be high, but irregularities in mating may reduce the number of young born—all of which is quite in keeping with the underpopulation phenomena explored by Allee and coworkers (see Allee, Emerson, Park, Park, and Schmidt, 1949:399–405). Nevertheless, the *lower* of the densities that permit *efficient* mating tend to show the *higher* rates of increase. For a good Iowa marsh having moderately low breeding populations, averages of 12 to 16 or even more young may actually be raised per adult female during a "normal" breeding season. For the same marsh having higher breeding populations, the net productivity per adult female tends to decline, until, at top-heavy densities, the females may successfully rear averages perhaps as low as two or three young, besides being subject to greater mortality themselves.

The inverse relation usually to be seen between population levels of adult muskrats during May and June (the principal breeding months) in Iowa

habitats and the rates of population gain by fall is in some ways reminiscent of the upper asymptote of the Verhulst-Pearl-Reed logistic curve, which is so widely applicable to population growth of organisms (Pearl, 1937; Allee *et al.*, 1949:301–315). The two methods of plotting data on coordinate paper overlap only in part. I have used them both whenever possible but have found that, for a species as subject to variable reductions between fall and the next year's breeding season as can be the muskrat in a cold climate, spring-to-fall rates of gain in relation to adult densities have revealed more of analytical value than have year-to-year population changes. Of course, numerical data on adult muskrat populations present during the main breeding season are not the easiest to obtain, whereas the fur trade may give us records on local or regional trapping catches and fall lodge counts usable as indices of the annual changes. We may often have a series of data sufficient to delineate a logistic curve if nothing more.

The lining up of a string of data points along either a curve of inverse gains or a logistic curve implies not only the self-limiting influence of the density factor but also compensating adjustments in rates of gain or loss and a certain stability of what Pearl (1925) called the "absolute base from which the law operates." Were it otherwise, pronounced variations in numbers of young born to a given population or in mortality suffered through specific agencies would show more *net* influence on populations than they commonly do, in that they would be more disruptive of conformation to evident mathematical patterns. In contrast with the Darwinian view of close interrelationships between population changes and what long have been assumed to be limiting factors, populations of muskrats (among other vertebrates) have repeatedly taken many big differences in vital statistics in stride.

The better the success of north-central muskrats in rearing litters born early in the breeding season, the sooner the year's breeding is likely to terminate (as in early or mid-summer, after the birth of one to three litters) and the greater may be the losses suffered by the young that do happen to be born late. Sometimes, the whole late-season (late June to September) production may simply be frittered away if most of the early-born young have been reared. Conversely, the poorer the success in rearing early-born young, the greater the likelihood of breeding being prolonged in compensation (as into August and September) and of unusually large proportions of late-born young surviving. This has been especially well studied in stream habitats in which nearly all of the young born up to mid-June were drowned in floods.

The extent and effectiveness of late breeding is plainly conditioned by the psychology of the muskrats. Diminishing of population tensions affords one of the best stimuli for reproduction. Where the animals live in superb but underpopulated habitats, we may get averages approaching four litters born per breeding female between April and August, with up to three-quarters or more of the young (late-born as well as early-born) being reared. Under

such conditions, the late breeding is stimulated not by death of the early-born but by the early-born being able to spread safely into places away from those frequented by the breeding adults.

Other evidences of automatic adjustments may be seen as different types of losses substitute for one another without perceptibly affecting the overall patterns. When, for instance, mink predation upon insecure young muskrats was severe, losses from other agencies (including other species of predators) tended to diminish; when mink predation was light or lacking, losses from other agencies tended to mount up and to do the eliminating of biological surpluses instead. Yet, when the muskrats introduced in the British Isles were successfully extirpated through deliberate campaigning (Warwick, 1940), we had an example of specialized predation that did not stop with elimination of a surplus. Past a certain point, the substitution phenomena in losses due to human and "natural" agencies and the stimulation of breeding through decreased population pressures no longer operated in effective equilibrium. Although ordinary fur-trapping or control-campaigning (as in central Europe) may cut muskrat populations down to levels below those expected to result "naturally" and thus be rated as having truly depressive influence, moderate intensities of human exploitation or persecution have their compensatory aspects. In other words, a great deal of the direct pressure that man applies to the species merely takes the place of "natural" agencies in the elimination of biological surpluses.

An abundant vertebrate and one occupying such a diversity of habitats and such an immense geographic range as the muskrat may present so many special cases as to make generalizing hazardous. It should still be proper to discuss the tendencies toward virtually endless counterbalancing between rates of gain or loss that may be expected to occur within the mathematical formula set for an area by the densities and psychology of the muskrats themselves.

The capacity for accommodation of environment for muskrats is hard to define, under that name or "threshold of security" or "biological base" or some other. It may show a noteworthy resistance to change, irrespective of many ordinary seasonal or year-to-year variations in water, food, and weather, but environmental changes may be of sufficient magnitude to affect a muskrat population.

Cattails or similarly favored foods may be killed by flooding, plant diseases, insects, livestock pasturing, or overutilization by the muskrats. Or, a muskrat marsh may deteriorate in time from no demonstrated cause, or perhaps as food plants fade out with ecological succession. Or, a food-poor body of water may dry up under exactly the right conditions to promote growth of cattail or bulrush seedlings, later to be partly reflooded and to turn, accordingly, into a splendid muskrat marsh in a single growing season. Events of this sort may decidedly change the status of the local muskrats, often with comparative suddenness.

There is plenty in both the resiliences and rigidities underlying population patterns that cannot be explained on the basis of our present knowl-

edge. A dominant self-regulating mechanism may be identified with the territorial or spatial intolerances displayed at least during breeding seasons if not at other times by very many populations of vertebrates (Nice, 1941). Among the markedly territorial of the vertebrates, we may detect a certain awareness of when "enough is enough," and, as social relations tighten, the individuals or groups possessing the equivalents of established property rights have a competitive advantage. Following the breeding months, social relations usually relax, and we may have observable phenomena (including loss rates) that look as if they might be considerably less a function of territoriality, *per se*, and more a function of the disadvantages of immaturity and of exposure to the dangers of unfamiliar places, to chance encounters with predators, to sources of infectious diseases, to climatic emergencies, accidents, etc.

One of the density phenomena that I have had trouble trying to make sense out of is the way in which big environmental and population changes have occurred in constituent parts of a large area without noticeably affecting the biological base holding for the area's muskrats as a whole.

In central Iowa, I had noted that local differences in numbers and productivity and in post-breeding movements might occur from year to year, to be offset by compensating differences elsewhere in the neighborhood. Some places looked crowded while others looked underpopulated, though the population of the observational area, considered as a unit, continued to conform to a year-to-year pattern.

Counterbalancing adjustments are particularly well indicated by records furnished me by the Hudson's Bay Company and the Manitoba Government. For an area of nearly three-quarters of a million acres near Cumberland House, Saskatchewan, the annual muskrat catches, plotted against time, 1930–35, defined the slope and both asymptotes of a logistic-type curve; then, following an engineering program to improve water levels for muskrats over a large part of this area, the 1939–46 catches again showed a tendency to line up along a logistic curve, but a curve having a distinctly higher base than that operative earlier. This much would seem to be a result of adding a large amount of muskrat habitat to what had been in the area during the preceding decade, but further engineering improvements in 1946 increased by about a third the acreage of productive marshland under management without appearing to have raised, up to 1951, the base governing the configuration of the logistic curve for the area's muskrats. Moreover, scrutiny of the details recorded for the constituent parts of the managed area left me with an impression of about every conceivable local variation in the year-to-year fortunes of the muskrats and the status of their habitats. Despite the local variations and the interplays of complexities, what looked like an over-all pattern somehow was maintained for years at a time, and similar trends are so well illustrated by the data from other Canadian areas that we cannot take for granted that they just happened.

DEPARTURES FROM "NORMAL" PATTERNS OF POPULATION ADJUSTMENTS IN MUSKRATS

For all of the tendency of a muskrat population to increase or to suffer losses according to a scale of rates that might be called predetermined by the density factor in relation to a given habitat, failures to conform are quite to be expected. Many of such failures are only temporary and readily understandable, as when droughts or epizootics may kill practically entire populations.

It should not be assumed that any drought or any die-off from disease necessarily has real depressive influence on the muskrat populations affected.

Droughts may not be of extreme deadliness to muskrats as long as the animals are neither deprived of the necessities of life nor forced into unsafe living routines. In the warmer months, muskrats generally withstand drought, even carrying on productive breeding, as long as water remains in their burrow entrances. Drought losses (or indirect losses associated with drought) occurring at times of year when biological surpluses are being eliminated in one way or another may be largely compensated as by decreased losses through other agencies, if the droughts are relieved before they progress too far.

Like compensations may attend losses through floods, violent storms, or epizootics, if these are not overwhelming; if light, the losses may be absorbed in the population adjustments; if heavy, the losses may or may not count in depressing a population, depending upon time and circumstances.

Should any of the numerous types of emergency or disease losses depopulate only parts of an area of muskrats prior to the end of a breeding season, overflow of adjusting animals from unaffected parts may soon refill the vacant quarters (always granting that conditions there again become favorable), sometimes so completely in a few weeks that drastic losses may have little or no net effect.

The distinction to be kept in mind in appraisals of the significance of emergencies and epizootics is whether the losses therefrom really reduced an otherwise secure population so that it remained below a level that it would have maintained or whether the losses merely gave otherwise doomed animals a chance to live that they would not have had.

Severe losses may also be compensated biologically after considerable delay. Efficient fur harvests removing half to four-fifths of muskrat populations between fall and spring surely may be said to depress the populations but they may also greatly relieve the tensions of the coming breeding season and thus promote more rapid recovery. Not only may the deadliness of fur harvests be partly or wholly offset through lessening the toll from fighting, subhuman predation, wandering, etc., and through stimulated reproduction, but it may also help to prevent epizootics from gaining headway and the more crowded of habitats from being destroyed by overuse.

But cataclysmic reductions of muskrats can occur, and unforeseeable depopulation through drought or disease on a county-wide or larger scale

may understandably upset calculations from an area's density figures and recorded history. Either drought or disease may dominate for long periods the whole life equation of muskrats, either locally or regionally.

The droughts of the thirties left muskrats alive only in the most exceptional habitats in the Dakotas, eastern Montana, and the southern Prairie Provinces, beside reducing the species over a much wider range during the driest of the dry years. During droughts, muskrats may die outright from thirst or heat, while wandering cross-country or while staying tenaciously in their home ranges. Spectacular losses may occur through the medium of subhuman predation, as mink, foxes, coyotes, dogs, raccoons, large raptorial birds, and other opportunistic flesh-eaters respond to the unusual availability of acceptable prey.

Drought also promotes trouble for the muskrats by concentrating them in the better habitats, to the accompaniment of increased fighting and restlessness (hence, increased vulnerability to enemies, as well) and miscellaneous complications; it may facilitate spread of infectious disease, as water may become foul or as sick animals may wander from puddle to puddle along a drying marsh bottom or stream bed; it may indirectly result in winter-killing, as shallow waters freeze to the bottom and seal off the food that would ordinarily be available in unfrozen water or mud. In the same way, a snowless winter in northern regions may allow very deep freezing of marshes. I have observed heavy mortality of muskrats under such conditions in Iowa and South Dakota, and Canadian correspondents wrote me that, due to lack of snow in the winter of 1949–50, the frost line sank five feet in northern Manitoba and Saskatchewan, all but wiping out the muskrats over a vast region of marshes and shallow lakes.

Since 1943, we have carried on in Iowa intensive studies of the epizootiology of a muskrat disease, of undetermined etiology but known over much of North America from its hemorrhagic and necrotic lesions and what can be great deadliness. Epizootics of this hemorrhagic disease (or of tularemia and conceivably of other virulent infections, at times) may make extensive areas nearly uninhabitable for muskrats for years, and so exert a population effect that is real, indeed.

The hemorrhagic disease may introduce highly confusing variables into population analyses, apart from questions as to numbers of muskrats killed thereby and the extent to which losses were or were not compensated. Sickening animals may have propensities for overland movements, for coming out on the surface of the ice in winter, for fighting among themselves, for being killed by predators, or for doing something else that could suggest things other than disease in the population picture. Undetected epizootics in muskrats—and clear evidences of disease mortality may easily be missed unless an observer is working in the right place at the right time and knows what to look for—have been wonderfully fruitful of public misappraisals of activities of predators.

Let us consider a rather common winter situation on a north-central marsh on which minks are present and muskrats are favorably situated with respect to food, shelter, water, and their own population densities.

We may see that minks repeatedly invade muskrat lodges and sub-surface retreats and that this may go on for weeks or months without having any lethal significance to the muskrats, whatever. The minks do show a preference for muskrat flesh and are usually able to kill muskrats if any subhuman predator can; but functional grown muskrats having environmental and psychological odds in their favor rarely need to let themselves be killed by minks. Particular lodges may be "bored" by minks a half dozen times or so in a winter, to be repaired or not repaired as the muskrats may choose; to be repaired immediately after the minks leave or weeks afterward, frozen plunge holes notwithstanding.

Habitual intrusions into muskrat retreats may reward the minks in other ways than by improving their chances for eating muskrat, but this will not be discussed here. Be it understood that a die-off from hemorrhagic disease may leave up to a dozen or more dead muskrats in a lodge and in northern latitudes these dead may remain refrigerated well into the spring. Responsiveness of minks to these "windfalls" of a favorite food may be manifested by unrepaired mink holes in lodges and burrows, latrines of mink scats made up of muskrat remains, of dead muskrats being dragged over snow or ice or shore, and heads, feet, tails, large bones, blood, and patches of muskrat fur scattered about. It is nothing exceptional to find minks inside of lodges cut open for examination, along with partly eaten muskrat carcasses. Intact carcasses may be strewn or lying in piles about the chambers, much as when muskrats actually are being killed in large numbers by minks during freeze-outs or drought crises.

Outdoors men having conventional ideas of the nature of mink predation upon muskrats and lacking knowledge of the workings of the hemorrhagic disease look at such scenes with conviction that the minks had been slaughtering. In my routine examinations of the viscera of muskrats retrieved from winter mink retreats during times of no evident emergencies, I have practically always found diagnostic lesions in muskrats found dead in groups and in a large proportion (though not all) of those found singly away from known foci of infection. Keeping informed as to current food habits of the minks has proved to be most helpful in discovering "hotspots" of the hemorrhagic disease and in tracing their spread, especially while the marshes are frozen. And, after the ice disappears in the spring, it is often possible to date the winter's dying for different sites from the sexual advancement shown by the more intact and better preserved of the victims.

Departures from "normal" patterns of population adjustments may also be expected in consequence of the "cyclic" or periodic influences next to be taken up.

ON THE EXPLORATION OF "CYCLIC" PHASES IN MUSKRAT POPULATIONS

Recent reviews by Elton (1942), Dymond (1947), Siivonen (1948), Kalela (1948, 1949), Franz (1950), and Christian (1950) illustrate the scope of modern investigations of cycle-like fluctuations in vertebrates. The literature on the subject is tremendous, and important contributions are scattered through everything from sporting magazines to museum reports. Attention

has been paid to "cyclic" and related phenomena principally in the northern parts of the northern hemisphere but not exclusively there, as may be seen from the work of Bodenheimer (1949) in the Middle East and of Wodzicki (1950: 139–141) in New Zealand.

Most of the seemingly periodic fluctuations in year-to-year populations of mammals and birds occur with something near either a three-to-four year rhythm or a 10-year rhythm. These two cycles have been considered by Vinogradov (1934) and by Siivonen (1948), in particular, as being related, but a great amount of the published evidence is still non-committal. Regional differences, species differences, and miscellaneous irregularities in fluctuations have beset studies of cyclic phenomena so much that some investigators have disclaimed belief in any periodicity at all while others have concluded that each species has its own local cycle.

As I see it, the outstanding source of confusion among reputable students of population cycles is overemphasis of fluctuations of animals or use of such fluctuations as the sole criteria of cyclic behavior. Variations in phases of ups and downs of populations may truly bewilder anyone looking for synchronies, and the exceptions are at best a hindrance to easy generalizing. I would state, furthermore, that fluctuations in numbers of animals from one year to another can be very misleading. Unfortunately, fluctuations furnish for many species about the only data we may have to go on.

It is a fair question to ask how a species may logically be classed as "cyclic" if it doesn't show periodic fluctuations. If the fluctuations aren't periodic, then just what do we look for?

We must look for common denominators, for synchronies (or lack thereof) in life processes over a sufficiently long span of years to bring them out if they are there.

We must also keep in mind that the numbers of animals comprising a population are resultants of a multiplicity of factors. Important in the regulation of numbers of a wide variety of vertebrates may be intra- and interspecific competition or the density factor, plant succession, human land use, climate, lethal emergencies, epizootic disease, and uncompensated predation, including that frequently taking the form of hunting pressure by man. In addition to these, some investigators, myself among them, think that a probable extramundane factor may affect living things in an essentially periodic manner. It is clear that the population impacts of at least the better defined of these factors vary with species, time, and place.

To the muskrat, a series of drought years, whenever it comes, may have greater population effect than anything I have ever observed to synchronize with any apparent "cyclic" phase. On the other hand, while Grange (1949) has convincingly emphasized the importance of forest growths following fires to the population status of the snowshoe hare in North America, my feeling is that something still unknown may be more of a master factor behind the "classically cyclic" fluctuations of the species.

In developing this thought, let us restrict our attention to the central and eastern regions of the continent, chiefly because of my greater familiarity

with the fauna of these regions and the population researches that have been carried on from Iowa, the Dakotas, and eastern Saskatchewan east to the Atlantic. During the past three decades, this geographical expanse has yielded some of the best long-term data we have on wild vertebrates.

For the times for which comparable data exist, the native grouse of the above regions fluctuated about as the snowshoe hares did, and, in the agricultural lands of north-central and northeastern United States, the fluctuations of the introduced ring-necked pheasant were in fair synchrony with those of the hares and grouse. The hares, grouse, and pheasants rather generally reached maximum levels in or about the years ending in ones and twos and minimal levels in or about the years ending in sixes or sevens. At the risk of oversimplification, it might be suggested that the hares, grouse, and pheasants may be more susceptible to the so-called "cyclic influence" than the other resident species of small to medium sized mammals and birds, which sometimes did and often did not fluctuate synchronously. Among the latter species we have the muskrat.

The muskrat has been classed as non-cyclic, as having a cycle inverse to that of the hares and grouse, as having a cycle running parallel to that of the "classically cyclic" species though preceding it in phase, and as fluctuating according to the usual "10-year game cycle." Elton and Nicholson (1942) ascribed—I think rightly—to long-term fluctuations in rainfall a great deal of the annual fluctuations in trappers' catches of Canadian muskrats between about 1850 and 1940.

Let us use the "key" years of the fluctuations of the hares, grouse, and pheasants somewhat as sighting points and see what appears to synchronize on the muskrat areas kept under observation.

The "cyclic low" of 1936–37 coincided with a terrific drought over most of my study areas. However, local muskrat populations escaping the consequences of drought showed depressed rates of gain and increased rates of loss in relation to their densities and the quality of habitat available to them—as also did bobwhite quail and a number of other species that were intensively investigated at this time (Errington, 1946).

The last "cyclic low," 1946–47, was not accompanied by any extremes of drought or other unfavorable weather conditions over my study areas, and the central Iowa muskrat populations that were most closely observed maintained themselves at moderate to fairly high densities. In fact, central Iowa had more muskrats during this "cyclic low" than during 1941–42, two years assigned on other grounds to the period of the "cyclic high"! Over thousands of square miles of muskrat range to the west and north of Iowa, populations of the species did decline in broad chronological agreement with population declines of the hares, grouse, and pheasants.

But the central Iowa muskrats, despite the lack of agreement between their fluctuations and those of the "classically cyclic" species, showed plenty of other evidence of being affected by a common depressive agency.

Data were obtained on the sizes of nearly 1,600 litters conceived by fully adult muskrats on the Iowa study areas. The averages were 6.6 for 78

litters in 1935; 6.3 for 43 litters, 1936; 6.5 for 41 litters, 1937–38; 7.2 for 68 litters, 1939–40; 8.3 for 79 litters, 1941–42; 7.5 for 300 litters, 1943; 7.0 for 362 litters, 1944; 6.9 for 108 litters, 1945; 6.4 for 65 litters, 1946; 7.2 for 118 litters, 1947–48; 8.1 for 83 litters, 1949; 8.0 for 211 litters, 1950. Most of the litters recorded for 1943 and 1944 were conceived by members of very top-heavy local populations, hence subject to density depression to a greater extent than were the sizes of litters conceived during the other years, for which densities of observed breeding populations were more nearly comparable. Even so, there is no reason to think that the average sizes of the 1943–44 litters were lowered by more than about a half muskrat because of unusual density effects, and, with these density effects dissociated, it may be judged that the annual differences in average litter sizes reflect something else.

The significance that I see in the changes in average litter sizes of the Iowa muskrats is the lining up of their years of minimum, intermediate, and maximum values with those of the corresponding changes in numbers of the region's hares, grouse, and pheasants. Actually, the changes in litter averages appeared not to affect the population levels of the muskrats, but they are of exceptional interest as a phenomenon, implying as they do an agency exerting some sort of depressive influence upon the physiology of the muskrats as well as upon the evidently more sensitive hares, grouse, and pheasants.

Relaxation of this depressive influence—whatever the latter may be—with the approach of the period of the "cyclic high" is suggested by data on the sexual status of young female muskrats trapped in the fall and early winter of the calendar years of their birth. Of 261 specimens of females of the year examined in November and December, 1936–38, all were sexually immature, as were all of 312 examined, 1939–40. Most of the specimens for 1936–38 were taken from places having late-season conditions about as favorable for late breeding as they usually are, but this was not true for the dry late summers of 1939 and 1940. For 1941–43, 788 females of the year were recorded as being sexually immature by early winter, but three specimens originally classed as small adults that had conceived single late litters were later thought to have been young breeding precociously; it is probable that more precocious breeding might have been shown by the samples for these years had the majority of specimens not been taken from populations of sufficiently high densities to discourage late breeding, generally, whether by full adults or by precocious young. For 1944–46, years accompanied by some to a great deal of late-season breeding on the part of bona fide adults, 1,028 females of the year showed no sign of sexual precocity. For 1948–50, of 702 females classed as young of the year that were examined among the trappers' carcasses, 24 had conceived a late litter in the year of their birth, and the preponderance of this precocious breeding occurred in 1950, coincident with what appears to be the beginning of a new "cyclic high."

The precocious breeding so far recorded has had trivial effect on the population status of Iowa muskrats, and I should not expect it to be important for the species at any latitudes at which late-born young do not have time to get a good start before cold weather. Like other reproductive changes accompanying changes in "cyclic" phases, such unusual precocity may give us a lead as to possible common denominators.

Returning again to sight along the "key" years of the hare, grouse, and pheasant fluctuations, we find the detailed data from the muskrat studies still more informative.

During the one "cyclic high" of 1941–43 that has been studied thoroughly on the Iowa areas, the muskrats occupying first-class marshy habitats were observed to tolerate maximum breeding densities up to the equivalents of nearly eight pairs per acre over sizable tracts, whereas, during the "cyclic lows" of 1936–37 and 1946–47, the breeding densities in similar habitats levelled off at around two or three pairs per acre, even when large numbers of homeless individuals were frequenting the peripheries. Both at favorable and unfavorable "cyclic" phases, the maximum breeding densities were patently determined by what the established residents would put up with, the savageness of the fighting and other manifestations of intolerance being similar to view for eight-pair densities in 1941–43 as for three-pair densities in 1936–37 and 1946–47.

These differences in tolerance are amply illustrated by the data on annual distribution of breeding territories of muskrats on the Iowa study areas. The spacing of territories with respect to each other showed a remarkable uniformity during the years of "cyclic lows"; in typical marshes and streams, they were established and maintained in attractive and rather unattractive parts alike; and, if the animals were motivated in their selection of territorial sites by anything beyond passable living requirements, it looked like a desire to get well away from neighboring territories.

Territories of other years, that is, all years falling in chronology between the "cyclic lows," not merely during the "cyclic highs," were spaced much more with reference to differences in quality of habitat. There might then be massing of three or four territories along a streamside corn field or in a clump of cattails in one corner of a marsh. Social intolerances may be witnessed during these local territorial concentrations, too, but the animals act as if disposed to adjust to considerable crowding in response to superior grades of habitat. At very high densities—say, equivalents of around a dozen breeding pairs settled per linear mile of a small stream—territories may be spread through the poorer as well as the better parts; but, at lower densities, the less inviting places tend to be left muskrat-vacant.

Population tensions in the muskrat are naturally accentuated during the main breeding season (April to July, in Iowa), following which is a period of minimal friction, continuing about up to the coming of hard frosts. But, post-breeding adjustments on the Iowa areas have not been any more exempt

from apparent "cyclic" influence than have the adjustments of the breeding season.

Although muskrats in late summer and early fall of any year may engage in local movements as food or water conditions deteriorate or as wintering quarters may be sought, such movements normally take place as orderly extensions of home ranges or along fairly definite avenues of travel. Except at times of great crises and "cyclic lows," these movements have been almost characterized by participating individuals acting in ways that could be called deliberate, purposeful, and intelligent—that is, for muskrats. During the two "cyclic lows" that we have studied, however, large numbers of muskrats have wandered over the countryside, footloose and desperate and vulnerable to all manner of dangers. In the fall of 1946, they engaged in exceptional wandering in central Iowa for no visible cause; moderate populations seemed comfortably situated in most stream habitats, yet the cross-country movement resulted in conspicuous mortality from motor traffic and put hundreds of strange muskrats into isolated bodies of water.

The excessive restlessness observed for Iowa muskrats during the "low" phases of the "10-year game cycle," together with other evidence, invites the thought that the species behaved at low to moderate densities during the "cyclic lows" in ways similar to those in which it behaved at very high densities during the more favorable phases. It may be presumed that physiological as well as psychological changes were involved and that the psychological changes were conditioned by the physiological.

Further inklings as to physiological changes accompanying changes in "cyclic" phases may possibly be afforded by observations as to times of dominance of the principal syndromes of the hemorrhagic disease.

The typical syndrome has been one of light to moderate intestinal hemorrhages, variable amounts of hemorrhage elsewhere in the viscera, as in lungs and kidneys, and a moderate to heavy sprinkling of necrotic foci over the liver. The interval between exposure and death for this syndrome has been slightly over a week, as shown both by the best field data and by laboratory experiments. This syndrome prevailed from the beginning of our intensive disease studies in the fall of 1943 through the spring of 1946.

From the fall of 1946 to about half way through the spring of 1947 and again in the spring of 1948, another syndrome prevailed, this one manifested by few or no liver lesions and by severe hemorrhages in lungs or intestines. We were unable to determine with accuracy how soon after exposure victims could be expected to die, but this syndrome could be seen to act rapidly, probably killing the animals in a few days and before many liver lesions had a chance to form. Many victims had the appearance of having bled to death from their hemorrhages in lungs, cecum, or rectum.

Between 1943 and 1948, the very young animals dying in the course of sweeping epizootics and picked up along with hundreds of larger victims rarely showed macroscopic lesions.

Epizootics continued, almost annihilating parts of populations that happened to be in line of spread, until early summer, 1950; but the prevailing syndrome had switched back to something like the typical one by the fall of 1948. By the fall of 1950, the disease had almost become nonlethal, manifested by liver lesions (even in the very young) and absence of hemorrhages.

By the fall of 1950, about the only muskrats still dying from the disease on the Iowa areas were at the sites of severe mortality of past years, hence thought to be individuals especially exposed to massive infection. The incidence of recognized sublethal infections in the general populations of central Iowa was also high by the fall of 1950. Of 446 muskrats trapped at large over one of our main areas in late November, 1950, 6.7 per cent were recorded as having liver lesions, as were 13.6 per cent of a lot of 59 trapped from the vicinity of an old "hotspot" of the disease at another place. Few muskrats active enough to be caught in traps showed lesions of the disease in years of great mortality and then usually very slight ones, such as a spot or two on the liver.

In considering our data on the epizootiology of the hemorrhagic disease on the Iowa muskrat areas, I would attribute the observed changes in syndromes to changes in resistance of the host animals rather than to changes in virulence of the infectious agent itself. Between 1943 and the middle of 1950, it did look as if infected population groups quite typically demonstrated their infection by dying. Some evidence of individual immunity was seen in both field and laboratory, but, as a rule, the spread of epizootics could be traced on a given marsh by the nearly complete annihilation of muskrats in disease-swept tracts. Twenty or more dead per acre were retrieved from places having hardly any left alive. Then, by the fall of 1950, the disease was as prevalent over the study areas as we have ever known it to be, yet almost nothing was dying from it.

Without implying that there might not have been changes in the immunity of our Iowa muskrats unrelated to the "10-year game cycle," it may be pointed out that the changes from moderate to severe to moderate again and then to benign syndromes of the hemorrhagic disease so far have appeared to be in chronological agreement with the changes in phase of the hare, grouse, and pheasant fluctuations of the north-central region.

My concept of an unknown factor underlying the apparent synchrony of "cyclic" manifestations is of something depressing certain life processes. Apparently, it may be far more nearly a master factor in determining abundance of some species than of others, but, on the basis of the evidence in sight, I would suspect that animal life generally may be subject to its influence, even if not to the extent of appreciably fluctuating in numbers. Years ago, in my gropings concerning possible mechanisms, I judged that essentially all the manifestations I had been classing as "cyclic" *could* reflect overstimulation followed by exhaustion.

Christian (1950) proposed that "cyclic" declines in mammals may have been due to exhaustion "of the adreno-pituitary system subsequent to the

stresses inherent in a high population level, severe climatic conditions, and the demands of the spring breeding season." He reviewed Selye's adaptation syndrome in this connection (see Selye, 1949: 837–867 and earlier writings). Christian also suggested in summary that the "length of the cycle is probably a function of the reproductive potential of the species involved, and this potential is applicable to that portion of the population in excess of the death rate for the development of peaks. The terminating factor is the attainment of a population above the carrying capacity of the environment," also the possibility "that longer daylight, decreased light intensity, and lower temperatures during the breeding season in the north maintain a higher reproductive potential in turn leading to more pronounced cyclic changes than in more temperate climates...."

I do not doubt the validity of the stresses discussed by Christian, but neither his nor the other writings of which I know that are preoccupied with fluctuations of the animals deal with the synchronies in manifestations of periodicities that seemingly may occur at times of no pronounced fluctuations in numbers.

My interpretation of the evidence from the muskrats is that stress has repeatedly accompanied both the attainment of top-heavy populations whenever occurring and the onset of "low cycle" years, whether the species were particularly abundant or not. There still appears to be something about the chronological "cyclic low," that is, the years of late decades ending in sixes and/or sevens in the north-central region, to cause moderate or even low populations to display the symptoms that we associate with overpopulation.

Despite the philosophical hazards and the lack of satisfaction of attempting to explain one unknown in terms of another, I do not see how we may, with scientific propriety, ignore the synchronies of symptoms that have gradually emerged from detailed population data.

Solar phenomena have not been neglected by investigators seeking correlations between periodic fluctuations of animals and possible extramundane influences. There would seem to be a connection between sunspot and weather cycles and so, indirectly, between sunspots and numbers of some kinds of animals. This notwithstanding, the sunspot cycles line up only part of the time with a particular "cyclic" phase shown by "classically cyclic" animal life. As MacLulich (1937) demonstrated for the Canadian snowshoe hares, sunspot cycles passed in and out of phase with the hare cycles if a sufficiently long span of years be considered: "Between 1856 and 1937 there have been eight rabbit cycles, but only seven sunspot cycles; the average length of the rabbit cycle was 9.7 years, but the sunspot cycle averaged 11.0 years."

A fair agreement between the low years of the "10-year game cycle" in the north-central region and the sunspot maxima may be seen for about the first half of the Twentieth Century (Stetson, 1947 p. 148). Alignments between the "high cycle" years for game and the sunspot minima look a good deal less indicative to me, notably in so far as some of the distinctly favor-

able "high cycle" years may be years of moderately abundant sunspots and as the beginning of "cyclic lows" may be years of no greater (or even fewer) sunspot numbers than were recorded for the "cyclic highs."

I do not know how to evaluate the possibility that the more recent data on sunspots as well as on populations may be the more reliable and that alignment of "cyclic lows" with sunspot maxima in recent decades may for this reason be more meaningful than non-alignment farther back. Without venturing farther outside of my own field of competence than I have any intention of going, I may say that I am unconvinced that sunspot cycles provide the answer for the particular 10-year periodicity that I think I see. At the same time, I do not wish to rule the sunspots out of the equation if they are really in it, though operating in ways beyond my knowledge.

DISCUSSION AND CONCLUSION: INTERCOMPENSATORY TRENDS AND BIOLOGICAL BALANCE SHEETS

Because of the prominence of automatic adjustments shown by the population data with which I have worked, as well as the prevalence of intercompensatory tendencies in the animal kingdom (Solomon, 1949), I feel most wary of computations undertaken to establish definite values in vital statistics of animals. This does not mean that I would discourage mathematical approaches to population problems, for I believe that advances may be made through them if undue assumptions be avoided. Neither does it mean that I am offering a blanket challenge to Chapman's (1931) "biotic potential versus environmental resistance" nor to Deevey's (1947) introduction to life tables for natural populations nor to any other scholarly work that has as its aim a more accurate numerical expression of biological phenomena.

My hope, in this writing, is to supplement the endeavors of biometricians by bringing out some of the things that must be taken into account in arriving at true-to-life formulas.

I question that the biotic potential of my Iowa muskrats could be expressed numerically with even a dependable prospect of 50 per cent accuracy. Not only may the average sizes of their litters vary with "cyclic" phases and intraspecific density (and other factors considered of much less general importance), but the numbers of litters born per adult female in a breeding season also depend upon degrees of crowding and the properties of the habitat. A population of breeding females may conceive or give birth to twice as many young as may another population of like densities and environmental status if the latter population merely happened to have been more fortunate in rearing its early-born litters. One may be reminded of the prompt renesting of many species of birds following destruction or abandonment of early clutches of eggs (Laven, 1940; Errington, 1942).

In another part of a balance sheet equation, we have losses from a variety of factors. Some are density-dependent and some are not; some are intercompensatory and some are not; but a substantial proportion in the muskrat and all other vertebrates with which I have worked personally are both

density-dependent and intercompensatory. A usually fallacious procedure is to assign values (arbitrarily or on the basis of research findings) to the different agencies of loss, to add up the values, and to consider the total arrived at as a figure reflecting the workings of Nature. Through this kind of calculating, bad distortions may be dignified by a semblance of scientific methodology that might deceive anyone not familiar with its analytical pitfalls. Unless they consider the automatic shifts taking place within life equations, well may anyone wonder that many thriving species can exist at all in the face of the enemies they have or the losses they suffer!

First, we must not forget that extraordinary mortality may offset itself by *stimulating* reproduction, more young being produced as a result of more dying. Likewise, that the total amount of loss suffered by a given population in a given time and place depends *in part* upon whether or not more individuals have been produced than the available habitat can securely accommodate.

Excess populations for an area may be numerically large or numerically small; they may comprise a large or a small proportion of the total number of individuals present. Sometimes, there may be no real excess population for an area, even for an abundant species. In marginal habitats, there may be no real security for a particular species, even when it may be barely represented. The "normal" range of variations in this respect is such as to warn us against improper assumptions of concreteness. A given muskrat population may appear to be remarkably secure at an average density of 15 per acre; another population may attain that level but be so top-heavy that three-quarters are candidates for elimination in one way or another; another may not be able to maintain itself at 15 per square mile, though temporary densities greatly exceeding this figure may occur.

The case histories of muskrat populations bespeak much regulation by formula, not just a formula in which values for the terms conveniently "stay put" so much as one operating along a sliding scale as populations rise or decline within the framework of toleration limits and environmental requirements of the species, "cyclic" phases, etc. Except in the sense that everything living must ultimately die, life equations of the muskrats tend to be replete with flexibilities.

A value assigned to a mortality factor that merely substitutes for another should not be considered the equal in population significance with one that genuinely brings about a net lowering of the population. What difference does it make biologically if a mink or a fox or another muskrat killed a young muskrat as long as death of that individual essentially made it possible for another young muskrat to continue living? The population impacts of a dominating emergency or epizootic are in an entirely different category. It is in mishandling of losses designated for specific agencies that one is apt to go farthest astray in balance sheet computations.

We are still in explorative stages so far as concern natural checks and balances in animal populations, and the muskrat data give us scarcely more

than indications as to what may be fundamental mechanisms. We can see that widely-held concepts as to limiting factors need a good deal of revision. The modern biometrician must be careful not to confuse with limiting factors those population by-products that occur incidental to, but with slight if any influence upon, the population status of a species. He must be careful not to confuse the *fact* of losses with the *effect* of losses.

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A GENERAL MECHANISM OF DIFFERENTIATION BASED ON MORPHOGENETIC STUDIES IN CILIATES

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INTRODUCTION

In the absence of conclusive evidence to the contrary, the concept currently held most widely is that somatic nuclei are equivalent genetically. To account for cellular differentiation on this basis, it is generally assumed that the cytoplasm is endowed with potential morphogenetic plasticity; that different local field conditions canalize given cells into different morphogenetic pathways; that the canalization process is self-reinforcing and entails a narrowing down of developmental potencies; and that genes are or are not effective explicitly, depending on specific substrate characteristics of given cytoplasm. In other theoretical formulations, developed particularly during the last decade, the genome is also assumed to be identical in all somatic cells, but the plastic properties of the cytoplasm are attributed in part to plasmagenes, variously defined.

Yet it has been clearly recognized by embryologists and geneticists alike, that progressive regional diversification of the genome might well occur. Possibly, such diversification could represent an important phase of differentiation. If so, mutational changes could probably not be involved, since normal, orderly sequences of development appear to require a less fortuitous foundation. But metabolic changes of the gene, possibly elicited by specific metabolic changes in the cytoplasm, admittedly could not be ruled out.

Recent studies on ciliates provide initial evidence for such nuclear changes, regionally correlated to differentiative changes in the cytoplasm. The experimental data are of sufficient scope, and their implications of sufficient significance, to warrant a first attempt at integrative interpretation. As presented in the following account, a general concept of differentiation emerges which is consistent with the conclusions of experimental embryology and physiological genetics, and has tentative applicability both to protozoa and to multicellular organisms.

SCOPE OF CILIATE MORPHOGENESIS

For descriptive purposes in the present context, it will often be convenient to distinguish between "morphogenesis," the new-formation of a particular morphological pattern, and "morphostasis," the steady state condition which maintains a particular pattern. Morphogenesis may either precede or succeed morphostasis; both involve differentiation. In ciliates,

both processes culminate in the visible expression of surface potentialities: it is primarily the ectoplasmic cortex which endows the infusorian with a particular diagnostic morphology. The principal morphogenetic events are regeneration and fission. Other developmental phenomena occur, *e.g.*, excystment, or unique structural transformations in a variety of parasitic forms. To a large extent, these entail differentiative sequences analogous in part to those of regeneration, in part to those of fission. In general, sexual processes *per se* have no immediate morphogenetic aspects.

Regeneration, elicited by structural or physiological injury, involves either direct replacement of injured or lost parts, or resorption of the uninjured parts and redifferentiation of an entirely new set of organelles. In fission, either a second set of organelles is developed in addition to the one already present, or the original set is resorbed and two new sets are differentiated; in either case, constriction then gives rise to two equivalent individuals.

It is now well established that each of the subdivisions of the ciliate body participates in morphogenesis. The essential role of the macronucleus in regeneration or fission has already been demonstrated by the early pioneers of experimental protozoology (Nussbaum, 1884; Gruber, 1886; Balbiani, 1889; Verworn, 1892; Johnson, 1893; *et al.*). Their observations have been confirmed for virtually every species subsequently studied. In contrast, the micronucleus has proved non-essential in a majority of cases. In some species, the presence of both micro- and macronucleus is apparently required for several or all phases of morphogenesis (data unreliable in certain instances; *cf.* review by Balamuth (1940).

As primary site of morphological expression, involvement of the ectoplasm in differentiation appears evident. Special functions of the cortical infraciliature have come to light through the pioneering work of Chatton, Fauré-Fremiet, Lwoff, and their collaborators (*cf.* reviews by Fauré-Fremiet, 1948; Lwoff, 1950), and through recent extension of their findings by Weisz (1951a, 1951b). Infraciliary kinetosomes are now known to act as local "organizers," uniquely determining the specific course of morphogenesis. This work has also resulted in the important realization that differentiative activity in ciliates, regardless of the verbal designation of the process as "regeneration," "excystment," etc., is analyzable in terms of a single fundamental pattern of events, in which kinetosomes play a decisive role.

The flowing endoplasm not only provides raw materials and energy sources for differentiation in progress, but through its initial metabolic condition also delimits the scope of differentiative activity. Thus the initiation or non-initiation of regeneration, for example, is determined in part by the amount and the types of reserve metabolites present (Weisz, 1948). Endoplasm, moreover, has an important, as yet only poorly understood function as a principal effector of form regulating movements and architectural rearrangements which characterize every morphogenetic event.

In the discussion to follow, evidence pertinent to the developmental function of both the nuclear system and the cytosome will first be reviewed. A conceptual synthesis of the conclusions will then be described.

THE NUCLEI IN DIFFERENTIATION

Both types of ciliate nuclei arise after fertilization from a synkaryon. During karyokinesis, micronuclei typically display chromosomes or chromosome aggregates, but macronuclei, dividing amitotically, do not. This generalization is probably justified, despite claims by some (*eg.*, Piekarski, 1941; Devidé and Geitler, 1947; Diller, 1948) that chromosomes can be seen in the macronucleus. Apart from the question of chromosomal organization, convincing evidence is available (*cf.* Sonneborn, 1947; also below) indicating the existence of macronuclear genes.

In its broad scope, the dichotomy of function of the two types of nuclei is well known. The micronucleus is primarily a germinal nucleus, largely non-essential except during karyokinesis. Somatic functions are principally under the control of the macronucleus. Four lines of evidence support this view.

1. Amicronucleate races are known for a large variety of species. Individuals of these races are normal, reproduce somatically for long periods, and remain capable of carrying out morphogenetic processes. Amicronucleate individuals are often less vigorous than micronucleate ones. The micronucleus presumably ensures optimal efficiency in the carrying out of somatic functions, but its presence is evidently not essential.

2. As already noted, the macronucleus alone suffices for regeneration in many species. In forms in which the micronucleus appears to be required in fission, but not in regeneration, the strong possibility exists that purely morphogenetic aspects of fission (that is, the differentiation of a double organism) are entirely under macronuclear control, and that the micronucleus is necessary primarily for divisional processes (that is, mitosis, constriction into two single individuals). In some species, however, both nuclei appear to be required for regeneration also. If the data are reliable, the possibility cannot be excluded that, in certain species at any rate, the micronucleus retains a somatic function.

3. The macronucleus, normally degenerating during or prior to fertilization, has never been found capable of acquiring a germinal role. In the mating of amicronucleate animals of Kahlia, Horvath (1947) finds small macronuclear fragments which give rise to new macronuclei. He regards these fragments as micronuclei. But since the organisms remain amicronucleate, and meiosis is not observed, his contention is unwarranted.

4. The most convincing evidence for the differences in nuclear function has been furnished by Sonneborn (1946). During the fertilization of *Paramecium aurelia,* the old macronucleus often fails to degenerate completely, but breaks up into a number of bodies, some of which later redevelop into normal macronuclei. Under such conditions, the synkaryon does not produce new macronuclei (Sonneborn, 1940, 1941). If such an animal, homozygous recessive for the killer gene *k,* is crossed with an animal homozygous dominant for this gene, the micronuclei arising in the former animal and in its progeny will be *K/k;* but the macronuclei, derived from the partial degeneration products of the old macronucleus, will be *k/k.* Under such

circumstances, the individual is found to remain non-killer. Evidently the phenotype is under macronuclear, not micronuclear, control. While micronuclear genes may remain active during vegetative stages, they are apparently unable to override the genic effects of the macronucleus.

This experiment reveals that each of the breakdown products of the old macronucleus contains at least one complete set of genes. In view of such evidence, Sonneborn (1947) concludes that the macronucleus normally contains many discrete subnuclei, each possessing a full genome. In this sense, the ciliate macronucleus may be considered "polyploid," as has been claimed by some, although "multinucleate" would be a more nearly correct term. It is not known whether individual subnuclei are actually polyploid or not. At any rate, most observers now subscribe to a polygenomal concept of macronuclear organization. In line with this view, amitotic macronuclear division implies the separation of two groups of subnuclei, achieved without a strictly quantitative karyokinetic mechanism. It may be noted that, historically, the probability of subnucleation has been implicit in numerous experiments on species in which the macronucleus can be fragmented by microsurgery: that single fragments retain the full potentiality of the whole nucleus has been discovered by the late 19th century protozoologists, and their work has later been amply substantiated.

Since the macronucleus arises from a diploid synkaryon, each subnucleus presumably is at least diploid. By comparing the relative amounts of nucleoprotein in the micro- and macronuclei of Paramecium, Moses (1950) estimates the latter to be approximately 40-"ploid." Such considerations point to a rapid development of numerous subnuclei, in the interval between anlage-formation and maturation of the macronucleus. Indirectly, evidence for this is provided by cytochemical observations on Blepharisma (Weisz, 1950a). These reveal a progressive increase in the concentration of polymerized DNA within the developing nuclear anlagen. The extent of DNA synthesis, delimited presumably by metabolic conditions, is also shown to be influenced by the competitive presence or absence of other macronuclear anlagen. Moreover, unless a given anlage can acquire a certain DNA content it cannot persist, but degenerates. These observations may indicate that a critical degree of subnucleation must be attained before a macronucleus can become fully functional.

Of the two nuclei, the macronucleus clearly plays the more fundamental role in processes of differentiation. The focal question to be asked is whether all subnuclei are or remain genetically equivalent, and whether they contribute equally to morphogenesis and morphostasis. Earlier evidence on the equivalence of macronuclear fragments (v.s.) appears to warrant an affirmative answer. However, recent experimental and cytochemical work on *Stentor coeruleus* and on *Blepharisma undulans* (Weisz, 1949b, 1950b, 1949c) leads to a different conclusion, yet does not refute the earlier data. In both species, micronuclei have proved non-essential for regeneration (Schwartz, 1935; Moore, 1924); both possess a single moniliform macro-

nucleus composed of a longitudinal chain of nodes. Experiments on the two species are parallel, and those on *S. coeruleus* will be reviewed.

During the early stages after fission, all macronuclear nodes are equipotential, and each supports normal morphogenesis and morphostasis. Nodal capacities have been tested by excising all but a single desired node from individuals at given vegetative stages, and by observing whether this node can mediate regeneration, or can maintain the differentiated stage of the animal. Appropriate control experiments have been carried out simultaneously. By these means it has been found that as the vegetative cycle progresses, the differentiation potential of posterior nodes gradually declines. The decline is the more pronounced the more posteriorly a node is situated. Thus approximately midway through the vegetative cycle, the most posterior nodes support only partial regeneration: a gullet is not formed, and the peristome develops incompletely; or in originally intact organisms, the gullet is rapidly resorbed and the peristome degenerates partially. These conditions persist until death from starvation supervenes. Prior to fission, near the end of the somatic cycle, posterior nodes no longer mediate regeneration at all, and intact individuals dedifferentiate the entire oral apparatus. At this stage, mid-nodes still support partial regeneration, and as at any other stage, anterior nodes are fully active. During the ensuing fission, the macronuclear chain coalesces, and the fusion mass later reelongates, renodulates, and splits into two chains prior to cytosomal constriction. Nodes thus newly formed are again fully active, regardless of relative position. It has been found that, no matter how the fissional fusion nucleus is reoriented or fragmented, those nodes, and only those, which later come to occupy posterior positions, always lose morphogenetic activity. Moreover, once decline of activity has begun, experimental relocation of a node into an anterior position does not halt the decline, nor does it effect a return of the former potential. Such repotentiation is apparently achieved only if a node coalesces with others, and new nodes are modelled from the mass.

These data have led to the following conclusions consistent with the results as a whole. (1) Fissional condensation of the macronucleus probably entails a pooling of all morphogenetically active nuclear agents into a fusion mass. During renodulation, these agents would be distributed more or less evenly throughout the macronuclear substance. (2) The specific presumptive location of a nodal portion of macronucleus is determined largely by chance. (3) The cytosome at different antero-posterior levels apparently has a differential effect on the nodes, allowing retention of full activity at anterior loci, but effecting a progressive decline more posteriorly. (4) The decline of nuclear potential is irreversible, but coalescence of nodes brings about a reactivation of the critical agents. Thus nodes newly formed during fission are equipotential temporarily, regardless of position.

A precise parallelism could be demonstrated between the experimental data and the cyclical metabolism of highly polymerized DNA. Cytochemical

methods have shown that the protein content does not vary appreciably from node to node or from stage to stage. The Feulgen reaction is equally constant in all nodes and at all stages. But application of Pollister and Leuchtenberger's (1949) critical methyl green technique reveals consistent variations in DNA concentration. Prior to fission, the macronucleus displays an antero-posterior gradient of concentrations paralleling precisely the morphogenetic gradient. Analogous correlations exist for all other vegetative stages. Supplementary experiments on hydrolytic breakdown and on extraction of nucleic acids, as well as Pollister and Leuchtenberger's interpretation of methyl green data, warrant the conclusion that decline of nodal potential is probably due, at least partly, to local depolymerization of thymonucleic acid; and that restoration of the potential during nuclear coalescence may be a result of repolymerization of the degradation products. It may be noted in this connection that Seshachar, (1950) emphasizing the chemical heterogeneity of the macronucleus in Chilodonella, is also led to infer the presence of different polymeric forms of DNA.

As pointed out above, parallel evidence, on both the experimental and the cytochemical level, has been adduced for *B. undulans,* and analogous conclusions are warranted. In this species, nodes destined to be inactivated are always situated near mid-body, in the posterior half of the organism. Unlike chromatin in *S. coeruleus,* which persists even when inactivated, decline of nodal potential in Blepharisma is accompanied by a gradual resorption of the affected nodes. Changes in morphogenetic activity can thus be gauged visually. Anterior and posterior nodes persist, and by augmented growth compensate for the loss of mid-nodal chromatin.

The "morphogenetic agents" with which these data are concerned may be equated to the macronuclear subnuclei: if the agents were argued to be subnuclear derivatives, the latter would have to be assigned precisely those properties that characterize subnuclei themselves (for example, spatial definition, initial completeness of genome, presence of numerous unit-genomes within the macronucleus). In substance, therefore, the data imply that in each somatic cycle, initial subnuclear equivalence gradually gives way to patterned genetic divergence.

In *S. coeruleus,* non-equivalence of macronuclear fragments is not likely to be detected unless specific attention is given to the exact site of fragment origin, and to the exact cycle stage at which a fragment is tested. Thus all earlier microsurgical examinations of macronuclear capacity, based on an essentially statistical premise, failed to reveal the changing potentials. Among numerous random experiments, a small number of cases would probably be found in which non-regeneration and death are due to nuclear insufficiency. But this causation would not be apparent, and such cases would merely be added to the general category of "experimental mortality." In *B. undulans,* nodal resorption has been described earlier (Young, 1939). The process has been thought to indicate macronuclear "purification." In a sense, this is a close circumscription of the present genetic implications.

In species in which the macronucleus remains permanently compact (for example, Paramecium), experiments analogous to those described above cannot be performed. Microsurgical methods can therefore not be employed to determine whether, at certain stages, genetic differences exist among the subnuclei, or whether subnuclear equivalence is maintained permanently. Conceivably, the latter possibility could be a result of the continued state of nuclear condensation: conditions such as are provided temporarily in the fission nucleus of *S. coeruleus* would exist constantly, and genomes would remain fully activated throughout the vegetative cycle. Either alternative is consistent with the conclusion regarding the equipotentiality of nuclear fragments in Paramecium, implicit in Sonneborn's experiments on macronuclear function. The fragments, when tested at the time of fertilization, are derived from a skeined reorganization nucleus which would be absorbed ordinarily. Even if local genetic differences existed, skein formation would obliterate them, as is the case in the nuclear reorganization of *S. coeruleus.*

For Stentor and Blepharisma, intranuclear genetic divergence is noted to occur in accordance with a consistent, predictable, spatial and temporal pattern. It has been concluded above that this pattern is imposed by properties of the cytosome. Thus before the implications of the nuclear changes can be examined further, the morphogenetic role of the cytosome must be evaluated.

THE CYTOPLASM IN DIFFERENTIATION

Among cytoplasmic organelles, the "infraciliature" of Chatton and Lwoff (1935) is of chief interest in the present context. The infraciliary complex is composed of subpellicular, ectoplasmic granules and fibrils, demonstrable visually by a number of silver techniques. Microanatomically, the basal granules of cilia form Chatton and Lwoff's "kinetosomes." A row of kinetosomes is paralleled on the right by an associated fibril, the "kinetodesma." The precise nature of the association, apart from the fact of proximity, is as yet undetermined. Fibril and granular row constitute a "kinety," and all kineties of the organism comprise the infraciliature.

The known functions of the infraciliary components may be summarized as follows.

1. Kinetosomes are self-reproducing particles, endowed with genetic continuity (Lwoff, 1950). They cannot arise *de novo.* Kineties increase in length by kinetosomal reproduction. Localized lateral reproduction of kinetosomes may occur, resulting in the formation of extrakinetal fields. If lateral reproduction takes place over the whole extent of the kinety, newly formed kinetosomes may organize into a new kinety, interpolated between two existing ones.

2. Kinetosomes are pluripotent morphogenetically. Directly or as intermediate agents, kinetosomes are required for the differentiation and maintenance of: trichocysts, flagellae, cilia, ciliary derivatives such as membranelles, cirri, undulating membranes (Lwoff, 1949, 1950); contractile vacuoles, gullet vestibule, holdfast organelles (Weisz, 1951a). Other

kinetosomal functions are likely to be revealed in time. Even at present, the list tends to bear out the conclusion that most, if not all, differentiations of the ectoplasmic cortex owe their origin and maintenance to the kinetosomes. (The granules constitute elements of the fibrillar system: this system might also be under kinetosomal control.)

3. Exercise of kinetosomal function is contingent upon the supply of endoplasmic metabolites (Lwoff, 1950). Raw materials and energy sources are required in differentiative activity, whether kinetosomes primarily direct the proper utilization of these substrates (synthesis of cilia), or whether they form building blocks in the emerging organelle (formation of trichocysts). In this connection, the ectoplasmic proximity of mitochondria (Fauré-Fremiet, 1910; Weisz, 1950c) and kinetosomes may possibly be significant.

4. Certain kineties are usually specialized in that they, and only they, normally participate in the differentiation of more complex organelles. Fauré-Fremiet (1948) discusses five general patterns among ciliates which exemplify this rule. In one of these patterns (displayed by *S. coeruleus*), all kinetosomes organized into kineties differentiate and maintain cilia, the most characteristic result of kinetosomal metabolism. But only those kinetosomes which are elements of kinety I, the stomatogenic kinety, can in addition initiate the differentiation of the more complex oral organelles (or the contractile vacuole, or the holdfast, Weisz, 1951a). Moreover, in *S. coeruleus*, kinety I alone has been shown to be the system of reference in relation to which the specific polarity of the entire organism is determined.

New kinetosomes arising by division within kinety I acquire the specialized kinetal potential. Unique morphogenetic capacities thus are retained permanently in the kinety.

5. In contributing to complex differentiations, kinetosomes within kinety I first reproduce. The original member of each pair of granules remains *in situ* and continues to maintain a cilium. Newly formed granules participate extrakinetally in other morphogenetic processes. In the differentiation of oral structures, for example, a dense "anarchic field" of kinetosomes is first laid down. The field then reorganizes into double rows of granules, representing the infraciliary framework of the presumptive peristome (Lwoff, 1949, 1950).

6. Kinetosomes and kineties are fundamentally equipotential (Weisz, 1951a). In *S. coeruleus*, any portion of kinety I retains the potentialities of the whole kinety. If kinety I is removed entirely, another kinety acquires the specialized functions. Moreover, within kinety I, every locus possesses a multiple potential. By choice of appropriate conditions, a locus can be induced predictably to differentiate either membranelles, or simple cilia, or a contractile vacuole, or a holdfast organelle.

7. These and related studies, as well as grafting experiments in this species (Weisz, 1951b), reveal the existence of an ordered hierarchy of "dominance" relationships among kinetosomes. In the presence of the kinetosomes of the oral organelles, for example, stomatogenic potentialities

of any other kinetosomes remain inhibited. But as soon as oral kinetosomes are removed, kinety I initiates stomatogenesis. Similarly, kinetosomes of kinety I exercise dominance over the kinetosomes of other kineties. In kinety I, terminal kinetosomes are dominant over those bracketed within the kinety. If two or more sets of oral organelles and kineties I are introduced into a unified graft system, kinetosomes of the largest oral region inhibit the stomatogenic potential of all other oral kinetosomes, and smaller oral structures dedifferentiate. Analogously, the largest kinety I dominates over other kineties, whether these had been specialized originally or not. These interrelationships clearly parallel the different degrees of structural and metabolic complexity of given organelles. From a purely quantitative standpoint, a larger organelle is evidently more complex than a similar smaller one. Also, in line with Lwoff's (1950) views, a greater organizational complexity may reasonably be postulated for kinetosomes maintaining membranelles, for example, than for those maintaining simple cilia. That this assumption is justified is shown in part by cytochemical tests on *S. coeruleus* and *B. undulans* (Weisz, 1949d, 1950b; also unpublished data). These reveal greater chemical complexity in the oral organelles than elsewhere, estimated either in terms of concentration of a single metabolite (tyrosine, phosphatase), or in terms of variety of metabolites (mucopolysaccharides specifically present in peristome).

Consistent with the foregoing considerations, the following general conclusions emerge with regard to the role of the infraciliature in morphogenesis and morphostasis. (1) In the normal organism, regional diversity of the explicit morphology is due to a divergence of the morphogenetic pathways followed by different kinetosomes. In view of initial kinetosomal equipotentiality, selection of a specific pathway must be influenced by the regional characteristics of the ectoplasmic microenvironment (also Lwoff, 1950). (2) This canalization process results in an acquisition, or an accentuation, of specific organizational characteristics within the kinetosomes, that is, the latter become differentiated chemically. (3) Continued maintenance of these characteristics in turn determines or perpetuates the regional specificity of the local environment. This provides further morphogenetic channeling stimuli for any newly formed, as yet undifferentiated kinetosomes produced in the vicinity. (4) Certain loci in the ectoplasm thus acquire greater organizational complexity than others. This is likely to be reflected in differential competition for endoplasmic substrates. Consequently, the observed hierarchy of kinetosomal dominance may be an expression of a parallel hierarchy of metabolic advantages. (5) The morphostatic condition, maintained in this manner, may be upset by renewed morphogenesis through a number of circumstances; for example, through structural or physiological injury, or through the cyclic acquisition of specific metabolic states incident to fission. In each case, actual or "physiological" removal of a group of kinetosomes vacates that level in the metabolic hierarchy which had been held by them. (6) Groups of kinetosomes next lower in the hierarchy may thus utilize the unclaimed share of

endoplasmic materials. As a result, rapid reproduction is made possible, and new kinetosomes are produced which initiate a resynthesis of the lost parts. Resynthesis remains in "correct" channels under the limitations of the specific properties of the microenvironment, and also through the inhibiting barriers imposed by the remainder of the metabolic hierarchy. (7) In the course of resynthesis, the generating kinetosomes gradually acquire the characteristics, and the metabolic status, of those infraciliary elements which are being replaced. Thus the formation of additional undifferentiated kinetosomes is slowed and finally halted. A *status quo* is attained eventually.

In substance, ectoplasmic differentiation appears to involve a highly autonomous series of reciprocal interactions between kinetosomes and the local microenvironment. The "directed" character of a given differentiation sequence probably has its foundation in a morphostatic pattern which either pre-exists (as in fission), or is residual (as in regeneration); and in the self-limiting properties of the morphogenetic process, without which morphostasis would be impossible.

INTERACTION OF MACRONUCLEUS AND KINETOSOMES

Kinetosomal autonomy in morphogenesis, and somatic activities generally, are ultimately dependent on macronuclear functions. Evidence for this is ample, and some of it has been set forth in preceding sections. Similarly, a general reverse dependence of nuclear activities on cytoplasmic functions is revealed by various well-known phenomena. For example, cytoplasmic injury, starvation conditions, or the attainment of certain body volumes, soon lead to nuclear changes (for example, coalescence, division, or increased output of secretions). Recently it has also been shown that kinetosomes, particularly those of the developing adoral zone and the anarchic field, influence the kinetics of the macronucleus, bringing about either the initiation and continuance, or the inhibition, of nuclear coalescence (Weisz, 1951a). Lwoff (1950) has emphasized generally that cytoplasmic aspects of differentiation precede the associated nuclear changes. These cytoplasmic influences, however, have no direct bearing on the genic activity of the macronucleus.

Yet certain considerations, and the results of experiments on *S. coeruleus* and on B. *undulans* (Weisz, 1949b, 1951a, 1949c), appear to indicate that given genic activity of the macronucleus might in fact depend on the functioning of specific kinetosomes. The pertinent data are as follows.

In Stentor, experiments testing the morphogenetic potential of macronuclear nodes have demonstrated the progressive development, in the course of a somatic cycle, of a predictable pattern of subnuclear diversity. It has been concluded that this change is due to a differential antero-posterior effect of the cytosome. What property or entity of the cytosome could be responsible? Since varying nuclear potential is expressed principally in terms of oral morphology, and since oral organelles constitute the major distinguishing feature of the anterior region, it appears probable that the

differential effect on the nucleus is due to the presence of oral components in one cytosomal region, and absence of such components from another. And as shown above, the essential elements of the oral organelles are the specialized oral kinetosomes.

In Blepharisma, similarly, one group of specialized kinetosomes is associated with the oral region, located in the anterior third of the organism (the peristome reaches almost to the anterior tip of the animal and the posterior peristomal limit is sometimes near mid-body, most often within the anterior third of the animal). Anterior macronuclear nodes, in juxtaposition to this oral region, persist throughout the vegetative cycle. In addition, a second group of specialized kinetosomes is probably situated near the blunted posterior end of the organism and is associated with the complex system of contractile vacuoles. As has been shown experimentally (Weisz, 1951a), the formation and maintenance of contractile vacuoles depends on the activity of specialized kinetosomes located in or near the free terminals of specialized kineties. In Stentor, vacuole and associated kinetosomes are located at anterior kinetal terminals, where they are in close proximity to the oral kinetosomes. In Blepharisma on the other hand, the system of contractile vacuoles, much more complexly differentiated than in Stentor (Moore, 1934), is situated posteriorly. It may be inferred that the generating kinetosomes derive from the posterior kinetal terminals. Thus posteriorly, much as anteriorly, persistence of macronuclear nodes appears to be correlated with the presence of specialized kinetosomes in the vicinity.

A series of experiments tends to substantiate the implications. If it were correct that oral kinetosomes affect anterior nodes so as to maintain full nuclear activity; and if decline of stomatogenic potential in posterior nodes of Stentor, or in mid-nodes of Blepharisma, were indeed due to the absence of specialized kinetosomes from posterior (or middle) regions: then anterior nodes transposed into posterior (or middle) regions should decline; and posterior (or mid-) nodes, transposed anteriorly before their normal decline has begun, should retain full activity. Experiments of this type, carried out on *B. undulans,* confirm the principle of the hypothesis fully. A strict correlation is evident between, on the one hand, maintenance of nuclear function and proximity of highly specialized kinetosomes, and on the other, decline of nuclear function and absence of specialized kinetosomes from the immediate vicinity.

These data suggest that highly specialized kinetosomes may specifically affect macronuclear nodes in the vicinity so as to allow full nuclear function to be retained; less highly differentiated kinetosomes do not appear to have this effect. What might be the nature of such kinetosomal influences? Similarly, how does the nucleus affect the kinetosomes? These reciprocal interactions may be circumscribed broadly by the following.

In the absence of direct contact, and in view of the interposition of endoplasm which is constantly flowing, the physiological connecting link between macronucleus and kinetosomes may be postulated to consist of

diffusible substances, carried in either direction by endoplasmic currents.

It is clear that, in *S. coeruleus* for example, inactivated posterior nodes are inactive only from the standpoint of stomatogenesis: such nodes still suffice to differentiate and to maintain simple cilia (Weisz, 1949b; cilia cannot be differentiated anew in a wholly amacronucleate fragment, as repeated observation has shown). Macronuclear subnuclei must therefore contain agents required for cilium synthesis, distinct from other agents mediating stomatogenesis. Again, partially inactivated nodes cannot maintain a gullet and the proper geometrical alignment of the peristome; but the nodes suffice for the maintenance of peristomal components. Thus the existence of agents for "peristomal components" is indicated, distinct from agents for "gullet and peristomal organization." Reasoning along such lines leads to the conclusion that subnuclei may exist in at least as many different morphogenetically active states as there are possible levels of kinetosomal function.

Two major alternatives arise with regard to the possible nature of these different states. First, subnuclei unable to mediate stomatogenesis, for example, may lack the genes or the gene-products requisite for this process. Nodal inactivation might, in other words, be equivalent to differential and progressive inactivation of successive gene-groups. Second, the different morphogenetic states of the subnuclei might be an expression of a quantitative phenomenon. Conceivably, several distinct levels of overall nuclear activity might exist, not due to differential activity of given gene-groups only, but due to altered effective activity of all gene groups. As a result, the quantity, or the potency, of the subnuclear product might be reduced. The first alternative presupposes that the different demonstrable steps in stomatogenesis are under the control of different genes, the second alternative that they are mediated by the same genes, which would act at different effective levels.

Data concerning the different degrees of polymerization of DNA are probably consistent with either view. However, the following consideration perhaps tends to make the second alternative the more plausible one. Maximal nuclear activity can apparently be maintained by different types of highly specialized kinetosomes. A posterior node of Blepharisma, probably maintained in activity through the agency of kinetosomes associated with the vacuolar system, is morphogenetically fully equivalent to an anterior node, maintained by oral kinetosomes. Either node can mediate the differentiation of both oral and contractile organelles, as well as of any other ectoplasmic structure. Thus it would appear that once a kinetosome has attained a certain degree of differentiation, irrespective of the specific path of its differentiation, it may exercise a "maintaining" effect on all active entities of the macronuclear subnuclei. If progressive nodal decline would occur through inactivation of specific genic groups, then a posterior node of Blepharisma should soon lose its stomatogenic and every other but the vacuolar potential; and an anterior node should lose all but

the stomatogenic potential. In actuality, however, both types of nodes retain their total potential. Thus, although present evidence is by no means conclusive, it nevertheless tends to indicate that a number of distinct levels of genomal activity may exist, each of which produces a distinct maximal morphogenetic effect. As noted above, these different levels of activity are characterized by different degrees of polymerization of DNA. The latter might be a reflection of different chemical states of genes themselves, or of degrees of ploidy, or of differences in the immediate genomal product, or of other related entities.

Nuclear effects would be mediated by the production of origin-specific substances carried in the endoplasm. Continued expression of a specific kinetosomal potency would then be dependent on the availability of a specific nuclear derivative. Variously differentiated kinetosomes in turn would release origin-specific substances in accordance with their degree of differentiation. The least specialized, that is, the ciliary, kinetosomes, mediate the retention of only the ciliary potential of the subnuclei. Membranellar kinetosomes effect the perpetuation of the nuclear potential to produce membranelles, and thus to produce cilia. Thus, if the genome is to persist at maximal effectiveness, at least a number of the most highly specialized kinetosomes must be present, or must be regenerated.

How could oral kinetosomes in Stentor maintain, for example, stomatogenic potential in anterior nodes, but not in posterior ones, considering that the "oral" kinetosomal factor would be distributed throughout the organism by the endoplasm? The experiments suggest that just after fission, when all nodes are still equipotential, anterior subnuclei, by virtue of their proximity to the oral region, might be the first to receive the kinetosomal agents, or might receive the largest share. Conceivably, this could establish an initial competitive advantage. Once gained, perpetuation of such an advantage would thereafter not depend strictly on proximity to the oral region. As posterior subnuclei are inactivated due to decreasing availability of the kinetosomal factor, antero-posterior competition would become increasingly unequal, and this would reinforce the initial disparity. In contrast, a similar disparity would not arise with regard to the potential for simple cilia, inasmuch as cilia are distributed over the entire ectoplasm. The correlated kinetosomal factor would thus be amply supplied to all parts of the nucleus; much as the nuclear factor for "cilia," produced in every subnucleus, would be available to kinetosomes in any part of the organism. In general, the hierarchic relationships among variously differentiated kinetosomes appear to be paralleled by similar relationships among the subnuclei. Initial kinetosomal, as well as subnuclear, equipotentiality is followed by correlative diversification of both, in a reciprocally reinforcing cycle of activities predicated by the existing morphological pattern.

The specific role of kinetosomal agents on the one hand, and of nuclear agents on the other, may be delineated broadly. It has been emphasized earlier that the autonomy of kinetosomes is potentially considerable, being

rooted partly in intrinsic organization, partly in reciprocal, self-directive interaction with the local micro-environment. Yet explicit expression of this autonomy is contingent on effective availability of endoplasmic substrates for differentiative syntheses. This suggests that the basic function of specific substances emanating from the subnuclei may be enzymatic, permitting proper substrate utilization on the part of the kinetosomes. Analogously, gene reproduction and the manufacture of genic derivatives require endoplasmic substrates, again suggesting an enzymatic role for the agents supplied by the kinetosomes. "Inactivation" of a subnucleus thus may signify inability of the genome to reproduce or to manufacture characteristic metabolites, leading either to polymeric degradation (as indicated for *S. coeruleus*), or to degradation and resorption (as in *B. undulans*).

DIFFERENTIATION IN PROTOZOA AND METAZOA

The analyses of preceding sections may be combined into the following concepts:

1. Regional diversity of the ciliate ectoplasm appears to be a result of reciprocal, self-limiting, self-reinforcing interaction between kinetosomes and the cortical substratum.

2. In certain species, regional ectoplasmic diversity produces a correlated regional subnuclear diversification, through the agency of origin-specific kinetosomal metabolites. Differential availability of the latter entails inactivation or non-inactivation of given subnuclei.

3. Consequent differential manufacture of origin-specific subnuclear derivatives reinforces the morphological pattern existing in the ectoplasm.

4. Obliteration of the nuclear pattern, as in fission, still leaves the ectoplasmic pattern operative as a "template" according to which the original nuclear pattern is remodelled in a subsequent vegetative cycle.

5. Regeneration of a "correct" ectoplasmic morphology, following its partial destruction, is substantially equivalent to the reestablishment of an ordered hierarchy among variously differentiated kinetosomes. This process requires the continued presence of at least a residual portion of the original hierarchy. The metabolic barriers which it imposes provide the necessary morphogenetic canalization. With the further provision of specific nuclear derivatives, patterned redifferentiation may occur.

Derived principally from observations on Stentor and Blepharisma, such a concept is probably applicable to ciliates as a group. Differences of detail are likely to be encountered. Thus in some species, a possible accessory morphogenetic function of the micronuclei is yet to be explained. In others, a permanently compacted macronucleus probably does not display regional subnuclear diversity. But all ciliates possess kinetosomes organized into particular morphological patterns. The present concept implies in essence, that under certain "decentralized" macronuclear configurations local changes in the genome can be produced by activities of the kinetosomes. Subsequent activity of the genome, altered in accordance to the

local morphological pattern, then reinforces that pattern. But existence of a specifically altered genome is not essential for the maintenance or the re-establishment of ectoplasmic morphology. As long as the necessary nuclear derivatives are supplied, directed morphogenesis and continued morphostasis are primarily a consequence of prior morphostasis.

Kinetosomes are noted to have central significance as points of intersection between the microenvironment/kinetosome cycle, and the kinetosome/genome cycle. Caspari (1950) has labelled these granules "visible plasmagenes." It is doubtful whether this designation is justified at present. All that can be safely stated is that kinetosomes constitute integral components of the cortex, elements which in large measure circumscribe the differentiative powers of the ectoplasm. Thus, in line with Weiss's (1950) hypothesis, they are perhaps better regarded as demonstrable groupings of particular molecular species which, together with other, so far non-demonstrable groupings, define given cortical characteristics. It is undeniably true that many aspects of kinetosomal function point up a number of cytogenelike properties. Yet these differ in at least one important respect from the properties of known plasmagenes. To date, none of the latter has been shown to affect essential morphogenetic or morphostatic processes: a Paramecium without kappa particles is no less a Paramecium than one containing such elements. Thus if "plasmagenes" are postulated to play a role in morphogenesis, plasmagene data actually available probably cannot be adduced as evidence. A "plasmagene" designation of kinetosomes in Paramecium or elsewhere, consequently has little merit, but adds considerably to verbal confusion. Indeed, the mechanism of differentiation here described alleviates the need to postulate *bona fide* plasmagenes for every organism. A cortical system endowed with greater or lesser autonomy, and a genome which is potentially plastic, suffice to account for differentiation.

Is it justifiable *a priori* to extrapolate results on ciliates to other protozoa, or even to multicellular organisms? Baker (1948) probably argues correctly that the polyploid (multisubnucleate) ciliate is not equivalent to the "cell" of other protozoa or metazoa. This argument implies, and probably no other argument can deny, that the ciliate organism is comparable to the whole metazoan organism. As Lwoff (1950) states so aptly: "A multicellular organism has differentiated cells; a ciliate has differentiated parts." The basic distinction between metazoa and single-celled protozoa on the one hand, and ciliates on the other, is clearly that in the former a morphogenetic "unit" happens to be co-extensive spatially and temporally with a cytological unit, a biochemical unit, a physiological unit, etc. In ciliates, the morphogenetic unit has no distinct cytological counterpart. But such a unit evidently exists; it contains some endoplasm, at least one macronuclear subnucleus, and a portion of the cortex.

Could the present concept be applied to differentiation in metazoa? The following more or less obvious considerations tentatively suggest an affirmative answer.

The major conclusions of experimental embryology point to the probability that such range of development as a cell possesses is implicit in potentialities of the cell cortex (Weiss, 1950), whether through the agency of visible kinetosome-like particles (for example, the basal granules of ciliated cells), or whether through invisible molecular ecologies. That the cortex always possesses a certain degree of autonomy is indicated by the fact that surface membranes are self-perpetuating organelles, endowed at least with somatic continuity. Cortical autonomy is paralleled by the manifestation of regional differences in cortical organization, apparent at the earliest stages of metazoan development and becoming increasingly evident thereafter.

After the first cleavage division, the total genome becomes progressively "decentralized." Each somatic cell nucleus formally corresponds to a subnucleus of the ciliate macronucleus; germ line nuclei, where these can be identified, are equivalent to the micronuclei of ciliates. There is no evidence that level-by-level "segregation of developmental potencies" (Weiss, 1939) might not be paralleled by a correlated "segregation of nuclear potencies," through a mechanism similar to that described above. Such a process might play a considerable role in metazoan differentiation. The concept is not inconsistent with genetic and cytological evidence.

Reduction of subnuclear activity to one of several possible levels has been noted to be followed, during a subsequent karyokinesis, by reactivation and an increased stainability of nucleoprotein. In metazoan cells, reduction of genic activity in given cells might equally be followed by reactivation, during mitosis or meiosis. In the latter, enhanced chromatin stainability is known to occur. In a post-mitotic cell, the genome would be fully active initially. As in the ciliate, however, such a cell would soon reacquire the original level of nuclear activity, through the limiting barriers imposed by the surrounding morphostatic pattern. But if the cell is cultured in isolation, unaffected by the original morphostatic barriers, reactivation of the genome would remain effective beyond karyokinesis. Modulated behavior would then be possible (to the extent that certain fundamental genes may no longer be active at all: cf. Mather's 1949, distinction between major genes, oligogenes, and polygenes). This might be the case for loose or migratory cells of certain connective tissues. Such cells, surrounded by relatively large amounts of tissue fluid and existing in environments which are rather non-rigid structurally, presumably are not too strongly under the influence of local morphostatic barriers. Presumptive gametes also mature in relative isolation, being expelled from the generating tissues. The reactivated genome would thus persist beyond meiosis, and this would contribute materially to the differentiation potential of the zygote.

SUMMARY

1. The known morphogenetic functions of the ectoplasm, the endoplasm, and the nuclear apparatus of ciliates are reviewed. The conclusions are integrated into a general concept of differentiation. The basic arguments are as follows.

2. *a.* The phenotype in ciliates is shown to be principally under macronuclear control. Evidence is presented to indicate that the macronucleus contains many discrete subnuclei, each possessing a complete genome.

b. Experimental and cytochemical data reveal that the subnuclei are equipotential initially. In certain species, however, differently located subnuclei undergo divergent fates during the course of a vegetative cycle. Genic function is retained at some loci, but reduced or lost at others. This occurs in accordance to a predictable regional pattern, concluded to be due to a differential effect of the cytosome. In the course of macronuclear reorganization during fission, equipotentiality of all subnuclei is again restored.

3. *a.* Explicit expression of the differentiation potential in ciliates is known to be under the influence of highly autonomous organelles, the ectoplasmic kinetosomes. It is shown that these granules are pluripotent morphogenetically and fundamentally equipotential. In the course of morphogenesis, given kinetosomes become differentiated chemically.

b. The local microenvironment is found to determine the specific pathway of kinetosomal development. Variously differentiated kinetosomes in turn impress various regional characteristics on the cortical ectoplasm.

c. Consideration of a variety of experimental data leads to the conclusion that a characteristic morphological pattern is maintained by metabolic barriers and limiting conditions which canalize different kinetosomes into an ordered hierarchy of functions. Directed morphogenesis following partial destruction of the pattern is then a result of the reestablishment of this kinetosomal hierarchy. A mechanism is outlined by which this may be achieved.

4. *a.* Evidence is reviewed which indicates that diversification of the subnuclear genomes is probably brought about by the regionally correlated diversification of differentiated kinetosomes. Conversely, the altered subnuclear pattern reinforces the particular ectoplasmic morphology.

b. It is concluded that these effects are probably mediated by specific (presumably enzymatic) agents manufactured both in kinetosomes and in the subnuclei. Through these agents, given kinetosomes control the level of subnuclear activity, and *vice versa,* in a reciprocal, self-reinforcing, self-limiting cycle.

5. The general implications of the data are discussed in the light of modern hypotheses bearing on problems of differentiation. These considerations suggest that the concept presented may have tentative applicability both to protozoa and to multicellular organisms.

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"PADDLE": A SEX-LINKED, RECESSIVE GENE OF TRIBOLIUM CASTANEUM HERBST[1]

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The objective of this paper is to record the presumed spontaneous occurrence of a new mutation for the flour beetle, *Tribolium castaneum.* This mutation is of some genetic interest in that it constitutes, so far as we know, the first reported instance of sex-linkage among the Coleoptera. It also has potential utility for population research—a utility enhanced both by the availability of several other genes now described for the genus and by the fact that a considerable body of knowledge exists relative to the population ecology of Tribolium.

Genes, and a variety of genetic phenomena, have been reported for the beetle families, Coccinellidae, Chrysomelidae, Curculionidae, and Tenebrionidae. In addition, Smith (1950) has recently summarized in some detail the cyto-taxonomy of 24 families of Coleoptera, and has shown (1950a) that *T. castaneum* has 9 pairs of autosomes, a small X chromosome, and a minute Y chromosome.

Tribolium, a tenebrionid, is represented in the genetic literature by discussions of two genes for *T. castaneum* and one for *T. confusum.* Park (1937) described the gene "pearl" (*T. castaneum)* as an autosomal recessive which, phenotypically, removes black pigment from the central ommatidia of the eyes. Miller (1944), working with the same species, dealt with two body color "strains." He showed that a mutant gene, when homozygous, changed the normal dark-red color to black. The heterozygote was intermediate between the two homozygotes, or dark reddish-brown. A rate-effect on metamorphosis was correlated with the presence of the mutant, namely: the larvae of the normal strain developed more rapidly when reared on a nutritious diet than did those of the black strain. Park, Ginsburg, and Horwitz (1945) described the gene "ebony" for *T. confusum.* The effect of this gene, when homozygous, is to alter body color from the red-brown characteristic of the species to a color that is essentially black to the eye. Genetic analysis proved that the gene was inherited as a simple, autosomal recessive. Ebony beetles also displayed a fecundity significantly reduced by about 10 per cent below that of normal forms.

[1]This investigation was aided by a grant from the Dr. Wallace C. and Clara A. Abbott Memorial Fund of The University of Chicago.

These three genes of Tribolium are useful as "population markers" in the sense that their respective, somatic expressions are readily diagnosed by inspection. They share in common the feature that they influence pigmentation. The new mutant, now to be discussed, also is quickly differentiable but differs in that it affects antennal morphology rather than eye or body color.

Before advancing proof of the hypothesis that this antennal gene is inherited as a sex-linked recessive, it seems advisable to contrast briefly the normal antenna with that of the variant. Reference to fig. 1 will be helpful in this connection.

The normal antennae of *T. castaneum* are distinguished by the facts that they are clavate; that the scape and pedicel are not markedly differentiated as to size or configuration; that eleven segments comprise the entire structure, and that the distinct club is formed by the distal three segments. The mutant antennae depart from these characteristics in several ways. First, and of prime diagnostic value, is the point that the club always shows some degree of fusion. That is to say, the sutures between segments do not complete their circumferential paths and fail, thereby, to effect movable articulations. There is some variation between individual mutants in this respect. The variation centers in the fact that the club can become progressively more reduced, even to a place where its first proximal segment is represented only by a minute vestige. The right-hand sketch of fig. 1, however, depicts the typical appearance and can be taken, so to speak, as the type-form of the mutant.

There exists also an interesting, but not understood, difference between males and females. Microscopic examination of 214 mutants selected at random from homozygous, recessive stock cultures showed that the females (112 in number) invariably possessed all the eleven segments characteristic of the genus. On the other hand, the males (102 in number) had lost two segments, thus giving them a total of nine. This is not a completely precise statement because in some males there was doubt as to whether a tiny segment-remnant did, or did not, exist immediately contiguous to the club. But, in any event, it is quite certain that males differ from females in having fewer segments, and that the modal number for males is nine while females always have eleven. Thus, it would seem that, in the embryogeny of the mutant, antennal imperfections increase from proximal to distal with the first segments of the flagellum not affected while the more outward segments, including the club of course, are subjected to greater teratology. This suspected process is apparently carried further in the male than in the female as suggested by the difference in total segment number. With a little experience it is quite feasible to separate mutants, whether males or females, from normal beetles by macroscopic inspection.

The mutant antennae appear paddle-like, and, in keeping with the custom of drosophilists who frequently seem to enjoy a homey nomenclature, we hereby designate this mutant of *Tribolium castaneum* as "paddle."

GENETIC EVIDENCE

Paddle first came to our attention in September, 1948, when, during the course of routine census examination of a mixed population of *T. castaneum* and *T. confusum*, a single male displaying this character was noted. Although the focus of our laboratory is ecological rather than genetic, we are reasonably alerted to the possibility of spontaneously occurring mutations. If an atypical beetle is seen, especially one whose morphological anomaly is bilateral, it is segregated to await genetic study. Preliminary assay soon showed that the paddle effect was inherited, and, after a stock culture had been established, a series of replicated, single-pair matings was initiated. All matings were made in glass vials containing eight grams of

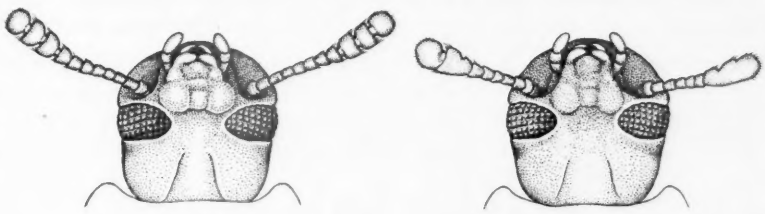

FIGURE 1. *Left*: Ventral view of the head of *Tribolium castaneum* showing normal antennae. *Right*: Same, but showing mutant-type antennae of male. (Drawings by Kenji Toda.)

"standard medium" (95 per cent sifted whole-wheat flour: 5 per cent Brewers' yeast), and maintained in incubators at a temperature of 29°C., and a relative humidity of 65 to 75 per cent. Using the notation "+" to denote type and "*pd*" to denote the mutant, the following genotypes were paired: (matings I and II first; matings III and IV, depending on the progeny of I and II, later).

I. Paddle male (*pd*/Y) × normal, homozygous female (+/+)
II. Normal male (+/Y) × paddle, homozygous female (*pd*/*pd*)
III. Normal male (+/Y) × normal, heterozygous female (+/*pd*)
IV. Paddle male (*pd*/Y) × normal, heterozygous female (+/*pd*)
V. Paddle male (*pd*/Y) × paddle female (*pd*/*pd*). (Stock cultures.)

The summed results from all replicates and all matings, and the ratios expected on the hypothesis that paddle is a single sex-linked recessive gene, are reported in table 1. Study of the table confirms the hypothesis and the following points may be arrayed in its support:

(1) Mating I yields only normal progeny in equal sex ratio. This follows expectation. The males are +/Y and the females are heterozygous +/*pd* as substantiated by the occurrence of paddle males among the offspring of Mating III. The excess of males (235) over females (202) is not a significant departure from 1 : 1 when tested by the point binomial (16.5 ± 10.4).

(2) Mating II yields 195 normal, heterozygous females (+/*pd*) and 185 paddle males (*pd*/Y). That the females are heterozygous is seen from the segregation of F_1

TABLE 1

OBSERVED PROGENY FROM FOUR MATINGS, AND PERCENTAGE PROGENY EXPECTED ON THE HYPOTHESIS THAT PADDLE IS SEX-LINKED AND RECESSIVE

Parental matings		Resulting progeny				Percentage of total progeny that are								Number of replicates
		Normal		Paddle		Normal				Paddle				
Designation	Genotypes	♂♂	♀♀	♂♂	♀♀	♂♂ (obs.)	♂♂ (expt.)	♀♀ (obs.)	♀♀ (expt.)	♂♂ (obs.)	♂♂ (expt.)	♀♀ (obs.)	♀♀ (expt.)	
I	*pd*/Y × +/+	235	202	0	0	53.8	50.0	46.2	50.0	0.0	0.0	0.0	0.0	8
II	+/Y × *pd*/*pd*	0	195	185	0	0.0	0.0	51.3	50.0	48.7	50.0	0.0	0.0	9
III	+/Y × +/*pd**	146	274	153	0	25.5	25.0	47.8	50.0	26.7	25.0	0.0	0.0	16
IV	*pd*/Y × +/*pd***	54	49	49	51	26.6	25.0	24.1	25.0	24.1	25.0	25.2	25.0	5

(* ♀♀ from Mating I. ** ♀♀ from Mating II.)

progeny in Mating IV into all four possible phenotypes. This finding agrees with the hypothesis. The sex and phenotypic ratio does not depart significantly from 1 : 1 (5 ± 9.7).

(3) Mating III yields the three expected segregants in the expected ratios of, respectively, ¼ : ¼ : ½, namely: 146 normal males (+/Y); 153 paddle males (*pd*/Y), and 274 normal appearing females, half of which are assumed to be homozygous (+/+) and half, heterozygous (+/*pd*). The observed numbers do not differ significantly from the calculated: for normal cf. paddle males, 3.5 ± 8.7; for all-males cf. females, 12.5 ± 12.0.

(4) Mating IV yields the four expected segregants in equal proportions: 54 normal males (+/Y); 49 paddle males (*pd*/Y); 49 heterozygous females (+/*pd*), and 51 paddle females (*pd*/*pd*). Again, the observed numbers do not depart significantly from the calculated expectation of 50.75.

(5) Mating V, or paddle male (*pd*/Y) by paddle female (*pd*/*pd*), has been maintained as a stock culture for over 25 continuous generations. It breeds true in that it produces, as expected, only paddle progeny.

It thus seems warranted to conclude on the basis of the above findings that the gene paddle is both sex-linked and recessive.

Finally, it is of some interest to examine, on a mean per vial basis, the total production of adult progeny within each of the four matings. These means are as follows:

Mating I: 54.6 ± 11.3 (C.V. = 58.6 per cent; d.f. = 7)
Mating II: 42.2 ± 8.1 (C.V. = 57.3 per cent; d.f. = 8)
Mating III: 35.8 ± 7.9 (C.V. = 88.3 per cent; d.f. = 15)
Mating IV: 40.6 ± 18.2 (C.V. = 86.0 per cent; d.f. = 4)

It is clear that no significant differences exist between any of the means. The greatest difference, that of (Mating I)-(Mating III), is but 1.4 times its standard error (18.8 ± 13.8). The production was also highly variable for all matings as attested by the coefficients of variability. These data cannot be taken as proving the non-existence of differential productivity since the experiments reported here were conducted, not to explore this question, but, rather, to obtain sex and character ratios. If desired, a more critical study could be readily designed to assay fecundity, fertility, and metamorphosis when crowding and intraspecies competition were minimized. But the mean values shown above do suggest that no striking trend in total adult production is correlated with parental genotype.

SUMMARY

A gene, designated "paddle," is described for the flour beetle *Tribolium castaneum*, and shown to be inherited as a single, sex-linked recessive.

The somatic effect of the gene is to cause varying degrees of fusion of the three segments constituting the antennal club. In addition, male mutant beetles show a reduction in the number of antennal segments from the eleven characteristic of the genus to a modal number of nine.

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A FURTHER ANALYSIS OF THE PANTOTHENICLESS MUTANTS OF NEUROSPORA

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During the last few years emphasis in the work on Neurospora biochemical mutants has been shifting from the use of these mutants as tools in determining pathways of biosynthesis to a study of the mutants themselves. Where previously it had been primarily a question of where the genetic blocks were located, it now seems more germane to inquire about the nature of these blocks. Since it has been assumed for many years, with good reason, that genes mediate chemical reactions through enzymes which they in turn control, the most feasible approach to the problem of genetic blocks is through a study of the enzyme systems in the mutant and wild type strains.

Initial attempts to distinguish enzyme differences between wild type and mutant, such as in the case of the adenineless mutant, 44206 by McElroy and Mitchell (1946), yielded inconclusive results. However, Mitchell and Lein (1948) investigated a tryptophanless mutant blocked at the reaction which couples serine and indole to produce tryptophane, and were able to show that cell-free preparations of wild type were active in this synthesis whereas those from the mutant were not. This result clearly indicated that the genetic block in question was due to the lack of an enzyme. It therefore provides support for the simple hypothesis that genetic blocks are due to the loss of enzymes *per se*. That is, loss of enzyme activity due to gene mutation is accompanied by the absence of the enzyme.

On the other hand, the work of Wagner and Guirard (1948) and Wagner (1949) on the pantothenicless mutants, 5531 and 34556 showed that the explanation of genetic blocks might not be quite so simple in all cases. It was found that in these mutants (which appear to be allelic or identical) the apparent genetic block is at the point of synthesis of pantothenic acid from β-alanine and pantoyl-lactone. The wild type is able to synthesize pantothenic acid from these precursors at a high rate *in vivo* in resting cell preparations but the mutant is not. When acetone powders are prepared from the mutant and wild type mycelia, these are found in both cases to be active in this synthesis. The mutant cannot carry out the synthesis *in vivo* under ordinary conditions of culture, therefore, but is able to do so under certain *in vitro* conditions. Obviously, the interpretation that genetic blocks are produced by simple loss of enzymes is not applicable in the

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case of the pantothenicless mutants, unless it is postulated that the acetone treatment of the mutant produced the enzyme artificially, as suggested by Horowitz (1949).

It is the purpose of this present communication to report the results of additional work on the pantothenicless mutants, and to discuss their implications in the analysis of how genetic blocks are produced. It was assumed that if the mutant mycelium possessed the enzyme for pantothenic acid synthesis, it should be possible to demonstrate its activity under certain *in vivo* conditions. This assumption proved to be correct as is shown below.

EXPERIMENTAL

Synthesis of pantothenic acid in vivo:

The initial approach to the problem of inducing synthesis of pantothenic acid *in vivo* was to modify the culture conditions. It had been previously shown that a number of Neurospora mutants such as the riboflavinless and adenineless mutants could be caused to grow in the absence of riboflavin and adenine by changing the temperature (Mitchell and Houlahan, 1946a, b). The vitamin B_6-requiring mutant, 299, grows in the absence of B_6 at basic pH's (above 6.0) but not at lower pH values (Stokes, Foster and Woodward, 1943). Under acid conditions the B_6-requiring mutant, 44602, will grow on minimal medium provided the ammonium content of the medium is increased (Strauss, 1951). Attempts were made to stimulate growth of the pantothenicless mutants, 5531 and 34556, in the absence of pantothenic acid by varying the pH, temperature and nitrogen content of the medium. No stimulation was observed even when suboptimal amounts of pantothenic acid were added. Therefore, it was assumed that no pantothenic acid was synthesized. None of the conditions was so drastic as to prevent the growth of the mutants in the presence of pantothenic acid.

Following these negative results an attempt was made to determine if pantothenic acid is synthesized by the mutant after growth has been initiated by the addition of pantothenic acid. There are a number of cases reported in which biochemical mutants have responded by synthesizing the required nutrilite after growth had been started in the presence of the nutrilite. The riboflavinless mutant of Neurospora has been shown to be one of these (Mitchell and Houlahan, 1946a).

The mutants, 34556 and 5531, and wild type, 5256, were tested in flasks containing 50 ml of standard Neurospora minimal medium, some containing in addition the pantothenic acid precursors, 0.004M β-alanine and pantoyllactone. The mutant culture media were made complete by the addition of 18γ of pantothenic acid per flask. The flasks were incubated at 25°C and 37°C and pantothenic acid assays were made each day on a set of flasks using *Lactobacillus arabinosus*. The results of these assays, which were essentially the same for both mutants at both temperatures, are given in fig. 1 for 34556 and for wild type over an eight-day period at 25°C. The dotted line in this graph represents the average dry weight increase over

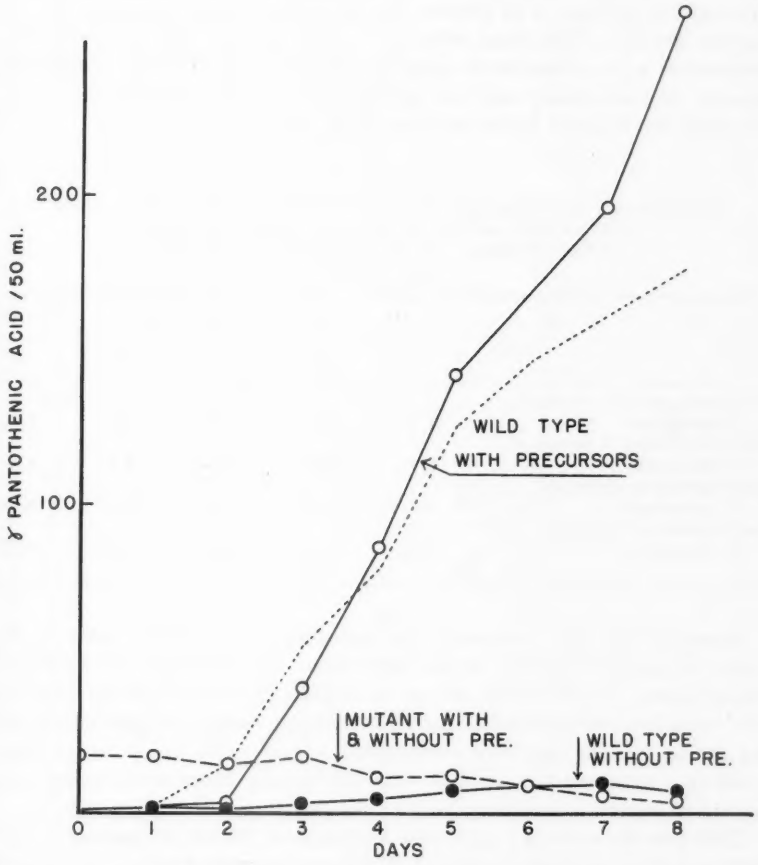

FIGURE 1. Production of pantothenic acid by wild type and mutants in standing culture.

the period observed. Actually assays were made each day for 13 days, but no change was noted in the amount of pantothenic acid present in the mutant culture media, whereas the wild type growing in the presence of the precursors continued to synthesize pantothenate, producing up to as much as 6 mg in 50 ml of medium. Wild type in the absence of the precursors produced no more pantothenate after 13 days than indicated in fig. 1 for an 8-day period.

To test the possibility that substances excreted into the medium by the pantothenicless strains inhibited the synthesis of pantothenic acid, mycelial pads were removed from some of the flasks on certain days, washed and incubated in the presence of 0.004 M concentrations of both precursors and

phosphate buffer at ph 6.5 for 24 hours. The results are given in table 1. Included in this table are the values for pantothenic acid synthesis by the wild type mycelium. It is evident that the intact mutant mycelium has no enzyme activity. The intact wild type mycelium decreases in activity for pantothenic acid synthesis on about the third day. In addition, comparable amounts of pantothenic acid are synthesized by wild type whether or not the mycelium is grown in the presence of the precursors.

TABLE 1

PANTOTHENIC ACID SYNTHESIZED BY UNTREATED RESTING MYCELIUM OF VARIOUS AGES AFTER GROWTH IN STANDING CULTURE. γ PANTOTHENIC ACID/MG DRY WEIGHT MYCELIUM PER 24 HOURS.

	Age of mycelium in days				
	3	6	7	8	13
Wild type grown in absence of precursors	8.7	4.1	2.4	3.0	1.8
Wild type grown in presence of precursors	8.9	5.1	3.0	4.9	1.5
34556 grown in absence of precursors	0.02	0.02	0.01	0.01	0.01
34556 grown in presence of precursors	0.02	0.01	0.02	0.03	0.01

Attention was next directed to the possibility of synthesis under conditions of vigorous aeration. It had been found that a culture of mutant mycelium grown in a five liter volume of medium under constant aeration for five days had accumulated 51 mg of pantothenic acid. This meant that 41 mg of the vitamin had been synthesized in five days, since 10 mg were used as a supplement to start growth. Subsequent experiments under controlled temperature conditions verified this synthesis.

The mutants and wild type were cultured in 500 ml of medium in one liter round bottom flasks immersed in a water bath set at 30°C. A constant stream of sterile air was bubbled through the medium in each flask throughout the course of the experiments by means of a glass tube which opened near the bottom of the flask. The culture medium for the mutant was supplemented with 1γ of pantothenic acid per ml. Pantothenic acid assays were made on a 10 ml aliquot of each culture every day for eight days. Fig. 2 presents the data obtained for 5531, giving the amount of pantothenic acid present per ml of medium. Flasks were also incubated at 25°C with essentially the same results. The volume of medium in the flasks did not vary more than 10 to 20 ml from the 500 ml original volume during the course of the experiment. It was kept within this range by saturating the entering air with water and replacing the assay aliquots with an equal amount of sterile distilled water after each withdrawal. On the eighth day the mycelium in each flask was removed, squeezed dry, weighed, and a small

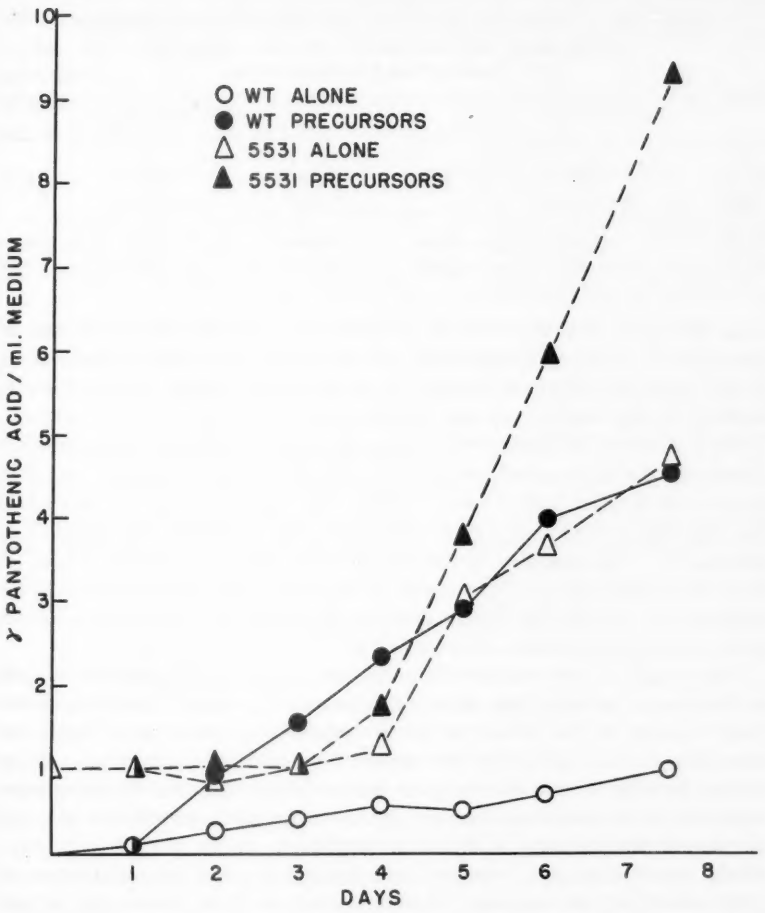

FIGURE 2. Synthesis of pantothenic acid by wild type and 5531 in an aerated medium.

sample dried and weighed. The remainder was blended with 0.1M potassium phosphate buffer at pH 6.5 and allowed to autolyze for 72 hours under toluene. The residue was discarded and the autolysate assayed for pantothenic acid. From this the pantothenic acid content of the mycelium was determined. The total pantothenic acid determined in the culture filtrate was added to this, after subtracting the pantothenic acid added originally to the mutant cultures. The total dry weight of the mycelium was determined and the amount of pantothenic acid synthesized per mg dry weight of mycelium was calculated. These results are given in table 2 for two independent runs using the above procedure. These values may be some-

TABLE 2

SYNTHESIS OF PANTOTHENIC ACID UNDER CONDITIONS OF AERATION. PANTOTHENIC ACID GIVEN AS γ PER DRY WEIGHT OF MYCELIUM AFTER 8 DAYS GROWTH.

5531 (mutant)			5256 (wild type)		
A Minimal only	B with precursors	B/A	A Minimal only	B with precursors	B/A
0.985	3.12	3.14	0.649	2.95	4.55
0.717	2.63	2.92	0.498	2.30	4.60

what low since pantothenic acid in bound form, particularly in the form of coenzyme A, is not assayable with *Lactobacillus arabinosus,* unless freed by the proper hydrolytic procedure. It is not known whether such hydrolysis occurred during autolysis of the mycelium.

Small amounts of resting mycelium grown under aeration were tested for activity in the presence of precursors and buffer by the procedure described previously (Wagner and Guirard, 1948). The results are given in table 3 for wild type, 5531 and 34556 cultured in the presence and absence of precursors. The activity of the mutant mycelium is evidently similar to that of the wild type. The activity of the wild type mycelium in this experiment is consistently higher than for the 8 day old wild type mycelium grown in standing culture. (See table 1.)

With regard to the observed activity for synthesis of pantothenic acid by the mutant cultures, the question arises as to whether there had been a back mutation to the normal at the pantothenicless locus, or a suppressor mutation. This possibility was tested by transferring small bits of mycelium from the mutant cultures into fresh minimal medium. No growth was observed in the inoculated flasks. On the other hand, growth was obtained if pantothenic acid was added as a supplement to the medium. It seems firmly established therefore that back mutation is not the explanation for the activity of the mutants. In this connection it is interesting to note that the pantothenicless gene seems to be a particularly stable one. Giles and Lederberg (1948) have performed extensive experiments to determine the back mutation rate of a number of biochemical mutants, and were unable to detect any for pantothenicless, although they found that other loci such as inositolless back mutated readily. The present authors have done similar experiments with pantothenicless and obtained similar negative results.

In the experiments previously reported (Wagner, 1949) in which pantothenic acid synthesis by the mutants was described, the evidence for the identity of the pantothenic acid synthesized by the mutant with synthetic pantothenate had been based entirely on the growth response of the mutants themselves and *Lactobacillus arabinosus.* While this is very good presumptive evidence that the two are identical it is not proof. Additional information as to their relationship was obtained by means of paper chro-

matography using a butyl alcohol-acetic acid-water mixture. The mutant-synthesized pantothenic acid has an R_f of 0.76 compared to 0.75 for synthetic pantothenate (converted to the free acid with oxalate before chromatography). The close similarity between the two R_f values makes it almost certain that the mutant produces pantothenic acid and not some other compound with similar activity.

COMBINATION MUTANTS INVOLVING PANTOTHENICLESS

Attention was next directed to the possibility of modifying the *internal* conditions of the mutant in order to suppress the phenotypic expression of the pantothenicless gene. The assumption was made that perhaps the enzyme necessary for uniting pantoyl lactone and β-alanine is inhibited by a compound or compounds produced by some other metabolic process.

In order to test the possibility that some naturally occurring compound synthesized by Neurospora might be responsible, a number of amino acids, vitamins, purines and pyrimidines were tested for inhibitory activity on the synthesis of pantothenic acid *in vitro*. None proved to be consistently

TABLE 3

SYNTHESIS OF PANTOTHENIC ACID BY 8 DAY OLD, RESTING, AERATED MYCELIUM

Strain	γ pantothenate per mg. dry weight mycelium per 24 hrs.
Wild type	6.8
5531	7.5
34556	7.7

inhibitory with the exception of cystine and betaine. Betaine was investigated further to determine its effect on the synthesis of pantothenic acid *in vivo* by wild type. In addition, tryptophane which definitely inhibits the growth of both wild type and pantothenicless was tested in the same way. Betaine proved to have no effect on wild type in concentrations up to 5 mg per 25 ml, but tryptophane showed a decided inhibition. In the absence of tryptophane, actively growing wild type mycelium produced 0.88 ± 0.06 γ of pantothenic acid per mg dry weight of mycelium from precursors in eight days, whereas in its presence (5 mg per 25 ml) only 0.54 ± 0.06 γ was synthesized per mg dry weight.

The pantothenicless mutant 5531 was crossed to UT 1, a mutant which when originally discovered in this laboratory grew slightly on minimal but required tryptophane for growth equivalent to wild type. Ascospores from this cross were picked at random and tested for growth on minimal, minimal supplemented with pantothenic acid or tryptophane and minimal with both. Ascospore isolations from single asci were not feasible since there was too large a number of inviable spores. Wild type, pantothenicless and presumed double mutant segregants were identified. However, no spores of the parent UT 1 strain were recovered. It was assumed that these were

possibly represented among the inviable spores. Cultures from viable spores which produced mycelium on minimal medium, but showed a stimulatory effect of pantothenic acid or tryptophane were assumed to be double mutants of UT 1 and 5531 and were tested by backcrossing to wild type. In a number of these both wild type and 5531 segregated in the same ascus sac but again UT 1 was not recovered. The results clearly showed, however, that 5531 was included in the double mutant strains. One of the double mutants, here designated as B3, was tested most extensively with respect to its growth requirements and ability to synthesize pantothenic acid. It is not only capable of synthesizing pantothenic acid, but its capacity for synthesis in the presence of the precursors and under conditions of aeration is from five to six times greater than the wild type and 5531.

UT 1, unfortunately, was practically sterile in crosses to wild type; only about 10 per cent of the spores resulting were viable, and all these proved to be wild type. It may be that UT 1 is not a simple point mutation, but a chromosome aberration. In addition to the aberrant behavior in crosses, UT 1 reverted to the wild type condition in so far as tryptophane no longer had any stimulatory influence on its growth. Because of this, and its sterility in crosses, experiments on this particular combination were discontinued.

The pantothenicless mutant was also crossed to UT39, an ultra-violet induced mutant which has been characterized in detail by Haddox (1951). UT39 grows only slightly (trace to 3 mg in 25 ml) on minimal medium during the first 72 hours, but after 72 hours its rate approaches that of wild type. In the presence of both phenylalanine and tyrosine together in equimolar concentrations of about 2×10^{-4} M, growth during the first 72 hours is almost identical to wild type. Tyrosine or phenylalanine stimulate growth alone, but not to as great an extent as in combination. Repeated backcrosses of this mutant to wild type have always resulted in a type of segregation characteristic of a single gene difference.

The double mutant obtained from the cross 5531 × UT39 was tested by backcrossing to wild type, and its constitution proven by finding that the mutant strains 5531, UT39 and the wild type and combination segregated in the expected ratios. The three types of ascus arrangements which occurred out of 16 dissected are as follows:

	Spores							
Ascus type	1	2	3	4	5	6	7	8
1	C	C	C	C	wt	wt	wt	wt
2	5531	5531	5531	5531	39	39	39	39
3	5531	5531	39	39	5531	5531	39	39

This double mutant has the following growth characteristics: on minimal medium it grows like the parent strain UT39; it is not stimulated by pantothenic acid, tryosine, or phenylalanine alone, but equimolar amounts of the latter two combined show definite stimulation (see tables 4, 5). Panto-

TABLE 4

GROWTH OF THE DOUBLE MUTANT (UT39, 5531) AND UT39 ON MINIMAL AND VARIOUS SUPPLEMENTS. MG DRY WEIGHT MYCELIUM AFTER 72 HOURS

Supplement	UT39	5531, UT39
none	5.0	1.5
tyrosine		
2×10^{-6} M	17	1.9
2×10^{-5} M	31	1.5
2×10^{-4} M	34	1.6
2×10^{-3} M	16	1.5
phenylalanine		
2×10^{-6} M	22	2.2
2×10^{-5} M	39	1.5
2×10^{-4} M	49	1.5
2×10^{-3} M	39	2.2
tyrosine and phenylalanine*		
2×10^{-6} M	19	1.5
2×10^{-5} M	30	6.5
2×10^{-4} M	40	2.7
2×10^{-3} M	21	3.6
pantothenic acid	4.1	1.6

*Range between 4×10^{-5} to 4×10^{-4} gives 20 mg or more growth of double mutant in 72 hours.

thenic acid and tyrosine combined have no effect on either UT39 or the double mutant, other than the expected tyrosine stimulation of UT39. The double mutant, then, shows a complete suppression of the pantothenicless phenotype, and in part the tyrosineless and phenylalanineless, since it does not respond to either of the stimulatory amino acids alone.

DISCUSSION

The original interpretation of the pantothenicless mutants of *Neurospora* as being due to the absence of the enzyme catalyzing the synthesis of pantothenic acid from β-alanine and pantoyl lactone (Beadle, 1945) is no longer tenable. It is now established that such an enzyme is present *in vivo* as

TABLE 5

GROWTH OF THE DOUBLE MUTANT (5531, UT39) AND UT39 ON MINIMAL AND VARIOUS SUPPLEMENTS. MG DRY WEIGHT MYCELIUM AFTER 84 AND 120 HOURS

Supplement	UT39		(5531, UT39)	
	84 hrs.	120 hrs.	84 hrs.	120 hrs.
none	10	40	3.2	40
20 γ/25 ml. pantothenic acid	8.2	40	6.2	39
2×10^{-4} M tyrosine	45	69	8.0	53

well as *in vitro* preparations, but is inactive under certain *in vivo* conditions. Alternative explanations for the genetic block in these mutants are therefore in order.

Since there is no other rational basis for hypotheses dealing with the primary or nearest observable action of genes evident at the present time, one must proceed on the assumption that genes mediate chemical reactions through enzymes. Starting then with the observation that the enzyme *per se* is present in this case one may interpret the results reported in this and previous papers by the following alternatives:

I. The mutation involves a gene directly concerned with the enzyme coupling β-alanine and pantoyl-lactone.

II. The mutation involves a gene controlling a reaction having nothing to do directly with the synthesis of pantothenic acid. This reaction results in the production of a compound which inhibits the enzyme catalyzing the synthesis of pantothenic acid.

Applying these hypotheses to the observed facts results in the following conclusions:

Considering hypothesis I first, we may conclude that any change brought about in the enzyme is not a simple quantitative change, since the synthesis of pantothenic acid by wild type and the mutant under proper conditions proceed at approximately equal rates. Nor, is there a simple qualitative change involving only an enzyme·changed in its affinity for its substrate. The extremely low activity of the mutant *in vitro* preparations at 25°C compared to the wild type, and to mutant activity at 30°C (Wagner, 1949) indicates a qualitative change, but there is no effect of increasing the temperature on the growth of the mutant in the absence of pantothenic acid. Furthermore, the mutant enzyme is active *in vivo* under aeration at 25°C. The change must involve the relationship between the enzyme and substances in the cell other than the pantothenic acid precursors. The enzyme in the mutant must be so altered that even though it is capable of carrying out the synthesis of pantothenic acid, it is prevented from doing so by the inhibitory activity of other substances. The affinity of the altered enzyme for the pantothenic acid precursors may be affected under certain conditions, but the more important result is that it is made subject to complete inhibition by other metabolites in the cell. These may be natural compounds such as proteins, amino acids, etc., which are involved in other phases of metabolism.

Using the first hypothesis, then, the activity of the *in vitro* preparations could be explained on the basis that an inhibitor or inhibitors are destroyed or removed in the process of preparation with acetone. The former would be more likely since concentrates prepared from the acetone washings do not seem to have any inhibitory activity on the synthesis *in vitro*. The demonstrated activity *in vivo* is more difficult to interpret, but one may assume that after an initial period of growth in aerated medium made possible by added synthetic pantothenate, the excess of oxygen or perhaps changes in CO_2 content bring about changes in certain phases of metabolism which

result in part in a decrease of the inhibitor. Dagley, Dawes and Morrison (1950) have reported that *E. coli* and *Aerobacter aerogenes*, when grown in unaerated medium, excrete amino acids (tentatively identified as histidine, aspartic acid, glutamic acid and alanine) into the medium which are easily detectable by paper chromatography without recourse to concentration of the filtrate. Cells cultured in aerated medium, however, only produce these amino acids in traces. The filtrate must be concentrated ninety-fold in order to get visible spots on paper. Accompanying this difference in amino acid excretion it has been found that the concentration of pyruvic acid increases during the active phase of growth of an aerated culture, but falls rapidly to zero after the stationary phase begins. In unaerated cultures the concentration of pyruvic acid attained is higher and the fall in concentration is not pronounced after the stationary phase sets in. Comparable but not necessarily identical changes in metabolism might be expected in the case of Neurospora as a result of aeration. Preliminary experiments conducted to test this have indicated that such is the case, but more work along this line is in order before any definite conclusion can be drawn.

The double mutant (5531, UT39), which grows in the absence of pantothenic acid, would by orthodox genetic terminology be described as a case of suppression of the 5531 gene by UT39. This is a good description of a condition, but not an analysis. A complete analysis of the suppression would require a more or less complete understanding of the inherited metabolic upsets in each one of these mutants, and we do not have this understanding at the present time. However, if one accepts the hypothesis that 5531 is incapable of producing pantothenic acid due to an inhibition, and that this inhibition is relieved in the presence of the UT39 gene by the reduction in concentration of the active inhibitor, a working hypothesis may be set up which may be of value for future investigations.

The second hypothesis given above is related to the first in so far as an inhibition is postulated. The essential difference is that the pantothenate enzyme is not altered, but that a natural inhibitor produced in excessive amounts due to mutation at another locus completely inhibits the enzyme at the higher concentrations. Both hypotheses, therefore, center around the relationship between an enzyme and its inhibitor. Altering the enzyme qualitatively or the inhibitor quantitatively should produce the observed effects.

It is not possible with the present data to differentiate between the two alternatives, or even to state that they are the only two possible explanations. Other hypotheses may be formulated which will explain the above described facts, but in the opinion of the authors the two given are the simplest, and most susceptible to further experimental investigation.

It must be noted in conclusion that the results obtained here with double mutants are not the only ones reported which are in conflict with the enzyme absence hypothesis as a general explanation of the inability of an organism to produce a particular chemical compound. Houlahan and Mitchell (1948),

Emerson (1948) and Mitchell and Mitchell (1950) have all described Neurospora double mutants of various types in which suppressors are involved. It is our opinion that proper combinations of mutant genes made by selected crosses between previously characterized mutants will result in a clarification of the concept that many biochemical mutants are due to internal upsets in the *balance* of metabolic systems. The object should now be to investigate the validity of this concept rigorously by combining unbalanced systems which complement one another and thus reestablish new balances. This is not heterocaryosis, but the synthesis of new balanced systems at a somewhat different level from the old. The evolutionary and biochemical implications of this concept are obvious.

SUMMARY

The pantothenicless mutants, 5531 and 34556, are active in the synthesis of pantothenic acid *in vivo* when the cultures are aerated. When 5531 is combined with the mutants UT 1 and UT39, the double mutants do not require pantothenic acid for growth. The bearing of these results on the analysis of genetic blocks is discussed.

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THE EFFECT OF NUTRITION ON THE PRODUCTION AND STABILITY OF AN UNSTABLE WHITE COLOR-VARIANT OF THE HY STRAIN OF SERRATIA MARCESCENS

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Recent studies with the Hy strain of *Serratia marcescens* have revealed a type of color variation which is not affected by mutagenic agents but which appears to be influenced by nutritional factors in the medium.

When suspensions of cells from typical Hy cultures were plated on peptone agar, color-variants were rarely seen and it was believed that the strain was relatively stable, but plating of the same suspensions on a synthetic ammonium citrate glycerol agar invariably gave a considerable number of white colonies which were conspicuously unstable and developed a few red spots after two or three days incubation. Because of the appearance of the colonies the variant was known as "speckled."

Variants of the speckled type were produced with great regularity by the Hy strain; 3-day old red colonies always contained about 1 per cent of cells of this type. Other spontaneous color variants were much less common and were relatively stable.

The speckled variants reverted rapidly to the pigmented type. When 3-day old speckled colonies were plated on synthetic agar they gave approximately ⅔ speckled daughter colonies and ⅓ red. Stable whites and intermediate pinks were not found.

Treatment of suspensions of red cells with ultra-violet light gave a great increase of other color variants (up to 30 per cent of the survivors gave colonies with heritable differences in pigmentation) but there was no change whatsoever in the proportion of cells that gave typical speckled colonies. Similar results were obtained by treatment with nitrogen mustard. The failure of mutagenic agents to affect the rate of production of the speckled variants, together with their high spontaneous frequency and their instability, clearly differentiated this type of variation from the others observed with the Hy strain.[1]

The number of speckled-type cells in synthetic cultures rose abruptly during the late lag and early logarithmic phases as found by plating ali-

[1]The common spontaneous, reversible, dark-red to bright pink variation previously described (Bunting, 1946) in the 274 strain has also been found to be unaffected by mutagenic agents.

quots on synthetic agar and observing the number of red and speckled colonies. This increase occurred even though the rate of multiplication of the speckled type in synthetic broth was lower than that of the red type. By means of marked strains it was clearly shown that the increased number of speckled-type cells did not come from the original white cells but from the red cells of the inoculum. During rapid growth in synthetic medium many of the progeny of the red cells had lost the ability to produce pigment.

No such increase in white variants was observed in media supplemented with peptone or yeast extract. Moreover, analyses of cultures by plating showed that populations of white cells inoculated into such media were rapidly changed into the red type. The evidence from the rate of change of the color types and from the behavior of mixed populations of marked strains indicated that selection was not responsible for the observed shifts but that in the presence of the supplement the white cells regained their ability to produce pigment and were changed into the red type. Even 24-hour colonies from speckled-type cells when grown on agar supplemented with yeast extract were bright red and contained a majority of cells of the red type, although sister colonies on synthetic agar were white and often failed to reveal the presence of any red cells at this early stage.

Until further data are available there seems no advantage in choosing between the various hypothetical mechanisms which can be conceived to account for the variations described above. The production of white cells during rapid growth in synthetic media suggests the loss of a cytoplasmic factor similar to kappa in paramecium (Sonneborn, 1946) or perhaps cytochrome-oxidase granules in yeast (Ephrussi, 1949); but the Serratia variants differ significantly in their ability to revert to the pigmented type when conditions are favorable. Actually the type of reversible change observed in the Serratia cultures has much in common with the mass phenomena described by Spiegelman (1950) for long-term adaptation to galactose fermentation in yeast. Further studies are in progress to identify the nutritional factors essential to the shifts in color types which have been observed and to elucidate the mechanisms by means of which they are accomplished.

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LETTERS TO THE EDITORS

Correspondents alone are responsible for statements and opinions expressed. Letters are dated when received in the editorial office.

ON MOSAIC INHERITANCE IN MAMMALS

Dr. W. E. Castle, in his article, "Are Mosaic Genes Semi-allelic?"[1], has commented on my article 2. He refers to the mosaic inheritance known in mammals as conforming only partially with the concept of semi-allelic genes presented in my article. In the "Japanese" rabbit, for instance, the black and red areas make a mosaic, and the whole behaves as a unit character which is alternative to either black or red. He interprets this to be due to "a reorganization of the gene molecule (or whatever it is that constitutes a gene) so as to include both alternative determinants side by side yet inseparable." It seems to me that the Japanese pattern is produced by the gene for black color (*E*) and the gene for red color (*e*) arranged in direct sequence on the same chromosome. These genes are so closely linked as to allow practically no crossing over. In the heterozygote with black, *E/Ee*, *e* is not shown, because of the presence of two *E*'s against one *e*. In the heterozygote with red, *e/Ee*, the mosaic character appears, because of the presence of two *e*'s against one *E*. The homozygote *Ee/Ee* is also mosaic, but in this the extension of the red area is probably more limited than in *e/Ee*. It is known that the Japanese pattern is highly variable, and it may appear almost like pure red in some cases. It is possible that such a Japanese has the formula *e/Ee*.

As for the mechanism of the bringing about such intimate relation between *E* and *e* on the same chromosome in the Japanese strain, I am inclined to postulate unequal crossing over between homologous chromosomes, one bearing *E* and the other bearing *e* which took place once many years ago. Since *E* and *e* are allelic, this seems to be the most plausible explanation. In the heterozygous state, *E/e*, *E* shows complete dominance over *e*. In the new relation on the same chromosome, *Ee/*, the dominance is no longer realized. Thus this perhaps represents another case of position effect. The many unexpected results encountered in breeding experiments on the Japanese rabbit,[3, 4, 5] as also those on the mosaic breed of guinea-pig,[6] seem to be understandable by assuming such an unusual interrelation between the genes concerned.

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June 18, 1951

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